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Assessment of biological particles

Title

Assessment of biological particles

References

International IDF Standard 148:A 1995, International Dairy Federation, 41 Square Vergote, B-1040 Brussels, Belgium.

Handbook of Fluorescent Probes and Research Chemicals, sixth edition, by Richard P. Haugland. Molecular Probes 1996 (ISBN 0-9652240-1-5)

Process and apparatus for counting particles, Lea;Tor E., Pedersen; Bjorn K., Naess: Harald K., US Patent 5428451

Method and apparatus for analyzing heterogeneous liquids, particularly for counting somatic cells in milk. Motolse, Guido, European Patent 0683395

Abstract

This invention relates to a method for the assessment of the number of particles in a volume of liquid sample material, the method comprising arranging a sample of the liquid sample material in a sample compartment having a wall defining an exposing area, the wall allowing signals from the sample to pass through the wall and to be exposed to the exterior, forming an image of signals from the sample in the sample compartment on an array of detection elements, processing the image on said array of detection elements in such a manner that signals from said particles are identified as distinct from the sample background, and, based on the signals from said particles identified assessing the number of particles in a volume of said liquid sample material.

Field of the Invention

The present invention relates to a method for routinely assessing properties of biological particles in a liquid samples. The properties being either the number of biological particles in a volume of a sample, or the morphological properties of biological particles such as size, or identification of one or more type of biological particles in a mixture of more than one types of biological particles.

Description of the Related Art

The principle of measurement of the present invention provides a major improvement in the assessment of biological particles, such as DNA containing particles, e.g. somatic cells or bacteria, or red blood cells, in a liquid sample material, such as milk, blood or urine, compared to the methods hitherto used for this purpose. One of these methods is flow cytometry, one such instrument available from Becton, Dickinson and Company, Franklin Lakes, which notoriously requires elaborate and high cost equipment, partly because of the high accuracy of flow rate necessary to give reliable results, and partly because the high sensitivity needed to detect the weak signals from the particles in question, in the relative short period of time the particle is present in the detector.

Another known method for the determination of somatic cells or bacteria in milk is based on the detection of signals from particles which are dispersed on the rim of a polished rotating disc, one such instrument available from Foss Electric, Hillerød, which notoriously requires elaborate and high cost equipment, partly because the accuracy in the assessment of number of particles is dependent on the physical shape of the thin film of sample dispersed on the disk, and partly because the high sensitivity needed to detect the weak signals from the particles in question, in the relative short period of time the particle is present in the detector.

One known method for the determination of somatic cells in milk based on spreading a film of milk onto a ribbon-like film which is then analysed by the means of a microscope, cf. European patent 0 683 395, an arrangement which requires a complex mechanical solution in order to work reliably.

Due to the relative high complexity and cost of production of the instruments used today, implies that most of the assessments of biological particles is carried out on in a laboratory, where skilled operators operate those instrument. The present invention offers substantial simplification of these assessments and makes it therefore possible to perform the assessment by operators without substantial skilled in the fields of technique. In particular it makes it possible to perform the assessment in the clinic or on the farm where the sample is taken, thus making the results of the assessment available for the user substantially immediately after the sample material has been collected.

The physical dimension of an instrument based on the present invention is also such that an instrument would be well suited for transport, thus making it possible for medical doctors, or veterinarians to transport the instrument on a location where the analysis is needed.

Summary of the Invention

This invention extends the capabilities of prior devices and methods to enable more simple and reliable assessment of biological particles in liquid sample material. The properties which can be assessed are the number of particles in a volume of the sample material, any morphological properties such as size or area of the particles, or the identification of the type of particle being analysed. In particular it is possible to assess more than one of these properties simultaneously.

At the same time, this invention allows these analysis to be carried out with the use of considerably smaller amounts of chemicals than normally are required to do these analysis. These chemicals are often considered hazardous, either to humans and other living organism or to the environment. Furthermore this invention presents a solution which minimises the exposure of any hazardous sample or chemicals used for the analysis by either allowing the analysis to be performed in a closed flow system or by the use of a sealed and disposable sample compartment, which contains all sample material and chemicals used for the assessment and allows save transport of the sample and any chemicals.

The high cost as well as the mechanical complexity of the instruments hitherto used for the routine assessment of the number of particles in liquid sample material has made the instruments impractical to use routinely under condition such as are normally present on dairy farms, on milk dairies, or medical clinics, or veterinary clinics. Such analysis are of great interest, for instance can a dairy farmer monitor the somatic cell count, or bacterial count of individual animal in order to follow the course of clinical or subclinical mastitis or infection, to control the cell count of the bulk milk delivered to the dairy, thereby minimising the use of antibiotics and prevent economical penalty often given when the cell count of bulk milk succeeds predefined limits.

Medical clinics are often in the need to know the count of one or more particles in blood, urine or other biological fluids such as somatic cells or bacteria, but since such analysis are often carried out in a central laboratory this often delays the response of such analysis due to transport of the sample.

It was found that this invention allows the analysis of various types of biological particles, such as DNA containing particles, red blood cells, blood platelets, yeast cells, bacteria cells, lipid globules, protein micelles, dust particles, or polymer particles, these particles normally found in liquid biological sample material such as milk, blood, urine, faces, salvia, inflammation, of either human or animal origin, or samples originating from the petrochemical industry, the pharmaceutical industry, feed industry, food industry or the like. The method is also well suited for the detection of

any other biological particle or fragments thereof, such particle being a part or a fraction of living matter and displaying properties which can be detected with the detection of electromagnetic radiation.

This invention is particularly suited for the assessment of the number of somatic cells in milk from human, cow, goat, sheep, buffalo or other animal. In particular this invention is suited for the assessment of the number of somatic cells in milk during milking by integrating the system with the milking equipment, either in-line where the measurement is taken substantially from the milking system and analysed by an instrument which is operated synchronised with the milking, or at-line where the sample is taken before, during or after milking and measured on an instrument in manual operation, in particular it is well suited to obtain an estimate of the number of somatic cells when the purpose of the analysis is to control the number of somatic cells in the bulk of milk delivered to the dairy, for instance by directing any milk which is found to have high cell count to a separate container or outlet.

Methods according to the invention are suited for the on-line or at-line assessment of the number of somatic cells in milk when the purpose is to establish information about the health status of animals, such as cows, goats, sheeps or buffaloes especially in connection with clinical or sub-clinical mastitis.

Methods according to the invention are suited for the assessment of the number of somatic cells in milk when the objective of the analysis it to generate information used in a heard improvement scheme, or when the objective of the analysis is to obtain a quality parameter used in a payment scheme. These analysis are normally carried out on a central laboratory, by the use of complex instruments.

An array of detection element can be utilised in combination with appropriate electronic components, to accomplish the assessment of biological particles in a sample material by placing a portion of the sample material in a sample compartment, in many embodiments of this invention the sample compartment being two windows of glass, or other transparent material, separated by a spacer with inlet and outlet which allows the sample to be replaced between measurements, in one embodiment of this invention the sample compartment is a tube, substantially circular, or substantially elliptical in profile. The presence of a particle will normally cause the signal from a detection element to deviate from a normal level, e.g. a base-line level, either towards higher signal intensity or toward lower signal intensity, but for the sake of clarity in the following it will be assumed that such deviation is toward higher signal intensity.

The present invention is based on the arrangement of the sample in such a manner that it extends over a "window" of a substantial area and detection of signals from the samples in the form of an "image" on an array of detection elements, the array of

detection elements comprising individual elements each of which is capable of sensing signals from a part of the sample window area, the array as a whole being capable of sensing signals from substantially all of the sample window area, or at least a well defined part of the sample window area.

As will appear from the following, the arrangement of the sample and the detection elements in this way will allow the determination of the number of the particles per volume in a much more simple and economic manner, while retaining a high accuracy of the determination. Also, as will be explained in the following, the use of an array of detection elements "observing" an exposed area of the sample makes it possible to use quite simple means for generating signals from the sample and quite simple and sensitive detection means.

Thus, an aspect of the invention relates to a method for the assessment of the number of particles in a volume of liquid sample material, the method comprising arranging a sample of the liquid sample material in a sample compartment having a wall defining an exposing area, the wall allowing signals from the sample to pass through the wall and to be exposed to the exterior, forming an image of signals from the sample in the sample compartment on an array of detection elements, processing the image on said array of detection elements in such a manner that signals from said particles are identified as distinct from the sample background, and, based on the signals from said particles identified assessing the number of particles in a volume of said liquid sample material.

The formation of an image of the sample on the array of detection elements may be performed by arranging the array of detection elements in close contact or substantially in close contact with the exterior of the exposing wall of the sample compartment, or by using an image-forming means, such as a lens, arranged in the light path between the exposing wall of the sample compartment and the array of detection elements.

The wall of the sample compartment defining an exposing area may be a flat or curved wall.

The sample in the sample compartment can be replaced by the means of a flow system, which is driven by a pump or a pressurised gas, preferably air. In many embodiments of the present invention the flow in said flow system is controlled by one or more valves which can adjust the flow speed of the sample.

In many preferred embodiments of the present invention the wall of the sample compartment is a plane wall, and the array of detection elements is an array extending in a plane parallel to the plane of the wall. However, dependent on the manner in which the image of the sample is formed on the array of detection elements, the

configuration of each of the exposing wall and the array may be designed in many different ways, such as where both the exposing wall and the array are configured as sections of a circular cylinder, such as where the exposing wall is convex and the array is concave with substantially the same radius, whereby they can easily be brought in contact or in substantial contact with each other, or where both the exposing wall and the detection array are concave, and a lens is used for formation of the image of the sample on the array. Many other configurations are, of course possible, such as where both the exposing wall and the array are sections of spheres, etc.

In many preferred embodiments of the present invention the sample compartment is a chamber which can easily removed from the instrument when a new sample or sample material is to be measured. Such removable sample compartment is preferably used for a limited number of measurements and preferably only one. Apart from allowing a more simple mechanical construction of an instrument with the absence of any flow system, one advantage of such removable sample compartment is to contain the sample in a closed container before, during and after analysis, thus allowing more save handling of hazardous material. In many embodiments of the present invention said removable sample compartment can, prior to the introduction of any sample material, contain one or more component or device used for chemical or physical modification of the sample prior to analysis.

Electronical devices or a computer equipped with suited software can be used to condition a signal which originates from any detection element used, preferably in such a way as to make the quantification of the signal from any detection element more reliable or less time consuming, for instance by converting one type of signal to another signal, and or by offering means for the amplification of the signal. Often it is preferred that the signal from any detection element is adjusted for any bias, and/or for any variation in sensitivity which might be present in the signals, this adjustment is preferably done by taking into account information from neighbouring detection elements, or by using similar information from previous measurement. Another useful property of such signal condition is the conversion of substantially analogue signal to a digitised value, which is better suited for further processing using a digital data processing system, such digitalisation could be either a threshold like activation of two or more output lines in such a way that the input level of any signal would cause a change the status of these output lines, preferably in such a way that the level of the input signal could be estimated. A preferred method of digitalisation is one which allows the level of the input signal to be converted to a number according to the binary number system.

It is often preferred that the digital representation of the level of any input signal produces substantially linear function, and in many preferred embodiments of this invention it is preferred that the digital representation produces substantially non-linear function, for instance a logarithmic function, such non-linear function being preferred when the dynamic range of the input level is high.

In some implementations of this invention, it is preferred to use a one dimensional array of detection elements, preferably included in one chip, the identification of a particle present in the sample which is measured, is done by comparing the level of signal from each detection element with a predefined level, or preferably to a level which is estimate on the bases of the signals from neighbouring detection elements, preferably on the bases of the signals from previous measurements, and if a signal is found to be above this discriminating level it is assumed that a particle was present, and a counter is incremented accordingly. Further it is possible to detect the presence of two particles measured at once for instance by comparing the intensity of a signal to a known or determined limits in such a way that signal above such limit indicate the presence of two particles, preferably more than one such limits can be used to identify any situation where three, four or more particles are present, or by constructing an empirical or theoretical relationship between the total number of particles present, and the possibilities of signals from two or more particles being detected simultaneously by a detection element.

To accomplish a reliable identification of particles using a described embodiment of this invention, it is often preferred that an optical system is used to focus any signal from the sample onto the detection elements, and further it is preferred that such focusing produces an image of a particle with an average size, which is of the same size as the detection elements used, and preferably smaller, such that the image of the entire particle is substantially within the boundaries of the detection element.

In many preferred embodiments of this invention, similar to the one described above using a one dimensional array of detection elements, or two dimensional array of detection elements, it is preferred that an optical system is used to focus any signal from the sample onto the detection elements, in such a way as to produce an image which is of the same size as the detection elements used, and preferably greater, the method being used to identify the presence of a particle taking into account also the extension of the particle in the dimension along the row of detection elements as well as the height of the measured signal from each detection element. Such embodiment of this invention allows the estimation of some morphological properties of the particles which are measured, such as the size. Also under those conditions it is possible to detect the presence of two ore more particles which are focused on substantially the same detection elements, for instance by classifying the signal intensity.

It was surprisingly found that a one dimensional array of detection elements, where the width of the array of detection elements was considerably greater than the height of each detection element, one commercially available from Hamamatzu (S3902-128Q), could be used for the assessment of the number of particles and thus enabling the detection of signals from a greater volume of the sample in each scanning of the detection elements. Further it was discovered that the use of focusing device which distorts the dimensions of the image, relative to the original, in such a way that for instance the image of a circle has a shape which is similar to an ellipse, also gave similar advantage as the use of detection elements with great height, and further it was found that the combination of both inventions gave further advantage with regard to increasing the detection volume.

The use of a series of one dimensional arrays of detection elements, preferably incorporated in a single chip, is often found to be useful in the assessment of biological particles present in a sample, one commercially available charge coupled device (CCD) is available from Sony (ICX 045 BL). Another array of detection elements suited for many embodiments of this invention is an image sensor based on CMOS technology which makes detection possible with the use of limited electrical effect, as well as offering on-chip integration with other CMOS based technologies such as signal condition and signal processing, one such has been demonstrated by Toshiba comprising 1318x1030 elements each about 5.6µm x 5,6µm in size using only 30 mW effect in use.

In the assessment of biological particles in a sample can be performed by treating each line of such two dimensional array of detection elements in substantially the same manner as an array of one dimensional detection elements.

Many preferred embodiments of this invention allow the simulation of high detection elements by the electronical or computational addition of information from two or more lines of detection elements into one array of information which is thereafter treated in the substantially the same manner as a single one dimensional array of detection elements, thus allowing substantially simpler and less time consuming interpretation of the measured information.

In many preferred embodiments of this invention the assessment of the number of particles in a first line of detection elements is based on any results, such as position and/or intensities observed in a second line of detection elements already being processed, thus allowing the correction of signals which extend across two or more lines of detection elements.

The inclusion of a focusing device for the focusing of a signal from the sample onto the detection elements in such a manner as to maximise the collection angle,

collection angle being defined as the full plane angle within which a signal is detected is in many situations been found to give improved condition for an assessment. Surprisingly it was found that such a wide collection angle, even to the extent that the objective used in the focusing distorted the aspect ratio of the image of any particle differently across the plane in which the detection elements were placed, or produced variation in the focusing across the sample being analysed, or reduction of the focusing quality was applicable in the assessment of the number of particles.

It has been found advantageous to make the assessment of biological particles in a sample by using a calculation mean, preferably a digital computer, one commercially available from Analogue Devices (ADSP 2101), is equipped with storage capacity which only can store information in amount substantially equivalent to a small fraction of the total number of detection elements, the assessment of the number of object being based on substantially real time processing of data, preferably in such a way that the measured information from each detection element, or a line of detection elements, or two or more lines of detection elements, is used for the assessment, substantially without any delay, such as a delay caused by storing the measured information.

At least one preferred embodiments of this invention allow the storing of substantially all measured information by the use of a first calculation mean, preferably a digital computer, before the processing of the information by a second calculation mean, preferably a digital computer, and thus allowing the measured information to be processed at substantially the same rate it is obtained, but with a substantial time delay between the measurement of any information and the processing of the same information, preferably this could be accomplished by only one calculating mean, preferably a digital computer, if such calculating mean were equipped with enough resources to accomplish the task.

When using a sample compartment used for the analysis of more than one sample material, for instance when the sample is introduced by means of a flow system, it is often found that one or more of the particles of interest, or fractions of particles, becomes adherent to the sample compartment in such a way that the flow used to replace the sample material is not capable of removing said adherent particles. Thus if said adherent particles are situated in a place which is exposed to the sensing device, it will be included in two or more observation although the sample has been substantially replaced between observations. In many embodiments of the present invention the influence of said adherent particles on the observation can be substantially eliminated by combining two observations in such a way that the result from a first observation is adjusted by the result from a second observation, said second observation being one of many observations taken prior to said first observation or a combination of more than one of many observations taken prior to

said first observation, preferably an observation taken substantially immediately prior to said first observation, said adjustment being a simple subtraction of said second observation from said first observation. The result of said adjustment then contains information where any objects present in said first observation have positive intensity, and any object present in said second observation has negative intensity and any object present in both first and second observation have substantially zero intensity. The task of any method used for the assessment of the number of objects or the determination of any morphological properties of an object is then to only treat those intensities which have substantially positive values. In a similar way it is possible to analyse the results of two or more observation taken from different samples from the same sample material by combining those observations as described above and subsequently to analyse both the positive and negative signals, for instance by treating all signals as being positive. In this way it possible to analyse 2, 4, 6, 8 or more observations simultaneously for instance in situations where the effort of analysing an observation is greater than the effort of making an observation.

This invention allows the sample material to be a substantially aqueous solution, or substantially organic solution, or a mixture of two or more inmiscible phases, some of which can be liquid, some of which can be solid and some of which can be a suspension, into which the particles of interest are suspended. In many preferred embodiments of this invention the sample material to by analysed has been modified or its chemical or physical properties substantially changed by either the addition of, or the removal of one or more components, or by introducing the sample to one or more chemical, mechanical or physical treatments prior to analysis. Preferably the effect of any such alteration or modification is the enhancement of any measurable signal used for the analysis, or a suppression of any interfering phenomenon, or it has the effect of prolonging the working life of the sample.

It is preferred in many embodiments of this invention that the signal which is detected is a photoluminescence signal, originating from a molecule, or a fraction of a molecule having fluorophor properties, naturally contained within or on the particle which is measured.

According to this invention the particles which are to be detected are often "coloured" with one or several molecules which bind to the particle, are retained within the particle, or otherwise interact with the particle, the effect of this "colouring" being the enhancement of any signal for the particle, or being the direct source of a signal which thereby can be used to detect the particle.

In many aspects of the invention the effect of the "colouring" is to cause, or enhance the attenuation of electromagnetic radiation such as visible light, or preferably

to cause, or enhance the emission of electromagnetic radiation such as chemiluminescence, or photoluminescence, e.g. fluorescence or phosphorescence, when exited with radiation which is substantially higher in energy that the emitted photoluminescence. One such "colouring" is the addition of Ethidium Bromide (EtBr) to the sample, where EtBr interacts with DNA material present in the sample, giving rise to fluorescence at approximately 605 nm when exited with light at approximately 518 nm (Handbook of Fluorescent Probes and Research Chemicals, page 145). This makes it possible, in the combination with the appropriate set of optical filters, to count a DNA containing particle where EtBr can interact with the DNA, such particles are for instance cells containing DNA, in particular DNA containing somatic cells or bacteria, such as those present in milk, blood or urine.

It was surprisingly found that it was possible to use concentrations of fluorophor which were substantially lower than those normally used in system, often less than 1/10th or 1/100 or even less than 1/1000, in particular this is advantages where added fluorophor exhibits relatively similar properties in free form as in bounded form, with regard to intensity and wavelength characteristics. As expected such condition inherently reduce any signal emitted from a coloured particle but surprisingly it was found that the ratio of the signal intensity in bound form to free form shifted in favour of bound signals. In particular it was found that a level of signal from fluorophor on free form in the sample which was comparable, and preferably less, in intensity to any random electronical signal (noise) and/or comparable in intensity to, and preferably less than, any other interfering signal was to be preferred.

Often the liquid, in which particles which are to be measured are suspended, is substantially at stand-still, where stand-still is defined as the situation where at least a part of the image of a particle does not move any more than it is contained substantially within the boundary of the same detection elements during one measurement period, preferably stand-still is defined as the situation where at least a part of the image of a particle does not move any more than it is contained substantially within the boundary of the same detection element during at least two measurement periods, thus allowing the detection of any weak signals which might indicate the presence of a particle.

In other embodiments of this invention, the liquid, in which particles which are to be measured are suspended, is substantially moving during measurement, in such a way that at least a part of the image of a particle gives rise to signal in two or more adjacent detection elements during one measurement period, or in such a way that at least a part of the image of a particle gives rise to signal in two or more adjacent detection elements during at least two measurement periods.

The liquid, in which particles which are to be measured are suspended, can be moving in more than one direction during measurement, for instance by controlling two sources of force, preferably which can be applied perpendicular to each other, thus giving the opportunity to move the sample in a predefined pattern, which can be used to improve the performance of any image processing device used to analyse the measured signal.

It is possible to perform more than one measurement and thus allowing a more accurate and/or sensitive assessment of the number of particles, for instance by measuring the same portion of the sample more than once and combining the results in order to improve the signal to noise ratio, and/or to measure more than one portion of the sample in order to increase the total number of particles which are counted to reduce the error in the assessment since the error in the particle count will normally follow count statistics where the relative error is expected to behave similar to one over the square root of number of counts.

In many preferred embodiments of this invention the number of measurements which are taken is defined by a real time estimate of the number of particles already counted thus performing relatively fewer measurements when the sample contains a high number of particles and relatively more measurements when the sample contains a low number of particles, preferably by defining an approximate lower limit for the total number of counted particles in such a way as an appropriate accuracy in the measurement is obtained.

It is possible to assess the biological particles in a relatively short time thus allowing a high number of samples to be analysed per hour, often more than 400, and even as many as 1000 or more analysis per hour. In many preferred embodiments of this invention an even higher number analysis per hour is achieved by including more than one measurement units which work in parallel in a single instrument.

This invention is well suited for the detection of electromagnetic signals and in many preferred embodiments of this invention the signals which are detected are attenuation of electromagnetic radiation, for instance caused by absorption or scattering, and in many preferred embodiments of this invention the signals which are detected are emitted from the particles or the samples, for instance emission of photoluminescence (e.g. fluorescence and/or phosphorescence) or raman scatter, and in many preferred embodiments of this invention the signals which are detected are caused by scatter.

Often more than one of the previously mentioned signals are detected simultaneously thus allowing more accurate or sensitive assessment of the number of particles or the assessment of any morphological property or to allow classification of

a particle present in the sample, preferably by the use of more than one set of detection elements.

A monochromatic device can be used to separate electromagnetic radiation into one or more wavelength components before one or several of these wavelength components are transmitted onto the sample either one at a time or more than one at a time, preferably when more than one wavelength component is transmitted onto the sample simultaneously the wavelength components are transmitted onto different portions of the sample thus giving an opportunity to obtain qualitative as well as quantitative information about particles in the sample. This is in particular of interest when the sample contains particles which respond differently to different wavelength components.

Light which can be transmitted onto the sample can be focused by a focusing system, comprising one or more lenses. The effect of such a focusing system is often to increase the effective efficiency of the light source. As light source it is possible to use a thermal light source, such as a halogen lamp, or a gas lamp such as a xenon lamp, a light emitted diode, a laser or a laser diode. It is often preferred to use more than one light source for the purpose of increasing the flux of light onto the sample, for instance by using two or more light emitting diodes. It is also possible to use more than one light source where some of the light sources have different electromagnetic properties.

A monochromatic device can be used to separate electromagnetic radiation emitted from, or transmitted through the sample into one or more wavelength components before such electromagnetic radiation is detected by a detection element, either in such a way that one wavelength is measured at a time or in such a way that more than one wavelength components are measured at a time. This is in particular of interest when the sample contains particles which respond differently to different wavelength components for instance when a particle is capable of emitting photoluminescence with different properties dependent on the nature of the particle. This effect can also be produced by the use of more than one type of light source which have different wavelength characteristics, preferably in combination with a monochromatic device.

In many preferred embodiments of this invention electromagnetic radiation, such as UV or visible light is transmitted onto the sample, in order to give rise to photoluminescence, in a set-up where the light source, the sample compartment and the detection elements all are situated approximately on the same axes, preferably where the sample compartment is situated between the light source and the detector elements. Surprisingly it was found that under these conditions it was possible to remove substantially all the excitation light which was transmitted through the sample

by means of filters, even in situation where high amounts of energy were used for the excitation. Further in many preferred embodiments of this invention it was found that it was possible to increase the efficiency of the electromagnetic radiation used for excitation by placing a reflecting device between the sample compartment and the detector which could reflect at least a portion of the energy transmitted through the sample compartment back towards the sample compartment, preferably where at least one of the surfaces which define the sample compartment was reflecting, preferably this reflecting device is one which has different reflectance properties at different wavelength, preferably in such a way that it is substantially transparent to the photoluminescence signal. One such reflecting device is a dichroic mirror.

It is often preferable to use one or several state of the art image processing techniques, such as 2 dimensional filtering or image identification, to assess the number of particles, or any morphological property of a particle.

With these and other objects in view, which will become apparent to one skilled in the art as the description proceeds, this invention resides in the novel construction, combination, arrangement of parts and method substantially as hereinafter described, and more particularly defined by the appended claims, it being understood that changes in the precise embodiments of the herein disclosed invention are meant to be included as come within the scope of the claims.

Brief Description of the Drawings

FIG. 1 illustrates one embodiment of this invention, particularly suited for the assessment of particles by the use of fluorescence.

FIG. 2 illustrates the effect of varying the initial concentration of fluorescent labelling dye.

FIG. 3 illustrates the possible removal of systematic bias by the subtraction of measured signals.

FIG. 4 illustrates an optical arrangement allowing collection of signals with a collection angel of approximately 40 degrees.

FIG. 5 illustrates an optical arrangement allowing collection of signals with a collection angel of approximately 70 degrees.

Detailed Description of the Preferred Embodiment

In the method of the present invention, the assessment of biological particles in a volume of liquid sample material is made by arranging a sample of the liquid sample material in a sample compartment having a wall defining an exposing area, transparent to electromagnetic signals emitted from the sample being exposed to the exterior, and forming an image of electromagnetic signals from the sample in the sample compartment on an array of detection elements, and processing the image formed on the array of detection elements in such a manner that signals from the biological particles are identified as distinct from the sample background, and based on the signals from the biological particles identified assessing biological particles in a volume of liquid sample material.

In the present specification and claims, the term "biological particle" designates a particle originating from, or found in living matter, such as somatic cells, red blood cells, blood platelets, bacteria, yeast cells, fragments of cells, lipid globules, protein micelles, plankton, algae or fraction thereof.

In the present specification and claims, the term "biological sample material" designates a liquid sample material of, often biological origin, or material where biological particles might be found, such as: specimen of human origin, specimen of animal origin, drinking water, waste water, process water, sea water, lake water, river water, ground water, food, feed or components of food and feed, milk or a milk product, blood or a blood product, urine, faeces, saliva, specimen from an inflammation, specimen from the petrochemical industry, specimen from the pharmaceutical industry, specimen from the food or feed industry, or product thereof.

The method allows a sample of the sample material to be analysed when practically all components in the sample material are present in the sample during the measurement on which the assessment is based. This is often practical when the liquid sample material is a biological sample material, since it is often associated with considerable difficulties to selectively remove one, or several, or substantially every component from a sample of sample material prior to analysis.

The array of detection elements can be arranged in such a way that they form a substantially straight line. When using a high number of detection elements they can be arranged in two directions in such a way that the detection elements form a series of substantially parallel straight lines, and often the array of detection elements is

arranged in one plane. This plane of detection elements is often arranged parallel to an inner boundary of the sample compartment.

The signal detected by the detection elements is normally an electromagnetic radiation and it is therefore preferable to have methods to transform those signals to measurable signal, such as voltage, or electrical current. Many such signals have a varying background signal, or bias, and it is therefore preferable to have methods which at least partly can eliminate those effects. This can often be accomplished by using a signal in one or several neighbouring detection elements as reference.

Another useful method is one where any varying intensity of the detection elements is adjusted, preferably by the use of results from one or several of previous measurements.

Arrays of detection elements are often made up of a high number of detection elements, and it can therefore be advantageous to reduce the number of measured signals prior to assessment, preferably without the loss of any significant information. One such method is to combine the signal from one or more detection element to one signal, for instance by combining 2, maybe more than 2 and even as many as 8 or 16 or 32 or more into one signal.

For the analysis of any measured signal it is often necessary to digitalise the signal, in such a way that a given intensity of any signal is transformed into a digital representation. This can be done by having a series of channels, were the information about which of these channels has signal which differs from the other channels determines the intensity, or even by having more than one of this channels forming a combination, preferably in a way similar to binary representation.

In order to increase the amount of electromagnetic radiation which is detected by a detection element, it is often preferable to use one or more lenses to focus the signal from the sample onto the array of detection elements. The magnification of such focusing can be different from 1/1, depending on the set-up of other components of the system, or the particles or sample material used. For instance can enlargement be practical when assessing morphological properties of a particle.

When the particles in question have dimensions which is comparable to the size of a detection element, it is often preferred to have magnification of about 1/1, thus focusing the image of any particle on any one or just few detection elements. This can under some condition give favourable detection of any signal.

When analysing particles which have dimensions which are comparable to, or bigger than the detection elements used, it is often advantageous to reduce the size of

the image of such particle, to a degree where the size of the image is comparable to the size of a detection element.

Surprisingly it was found that the aspect ratio of an image can be considerably distorted on the array of detection elements, without that having considerable negative effect on the assessment of particles.

The collection angle of a focusing arrangement used can have effect on the intensity of any signal collected on the array of detection elements. When high sensitivity is needed it is therefore practical to increase the collection angle. The preferred size of the collection angle can also be determined by other requirements which are made to the system, such as focusing depth.

The size of the detection elements determines to some extend its sensitivity. In some applications it is therefore of interest to have detection elements of size of about 1 μm^2 or less but in other applications a size of 100 μm^2 or even 1000 μm^2 or more is desirable.

The aspect ratio of the detection elements can be important in the collection of signals for the assessment of particles. A ratio of about 1/1 is some times preferred, but under some conditions it can be preferably to use ratio different from 1/1, even as low as 1/10, 1/100 or even lower, in particular when this facilitates detection of signals from increased volume of any sample, thus allowing simultaneous assessment of more particles.

Storage capacity, for instance used for storing information about measured signals from the detection elements, is often one of those components which have considerable effect on the cost of production. It is therefore of interest to be able to perform the assessment of particles without substantial any use of such storage capacity.

On the other hand, it is often difficult to accomplish assessment without the use of any storage capacity, but preferably the amount of such storage capacity should not be more than what is needed to store the information from all measured detection elements, preferably where only a fraction of the information can be stored.

Other, more complicated aspects of the assessment of particles, can require the use of considerable amount of storage capacity. In this aspect it can therefore be necessary to have storage capacity which can store more information than is collected in one measurement of the detection elements used.

A sample compartment, containing the sample being analysed, arranges preferably as much sample volume as possible in such a way that it can be exposed to the array of detection elements, thus allowing the analysis of many particles

simultaneously. One method for accomplishing this, is to define the thickness of sample compartment in a direction which is not parallel to the plane of detection elements, thus increasing the effective volume per are of sample compartment exposed to the detection elements. The thickness can be as much as 100 µm, or even more than 1000 µm, the optimum thickness often being determined by any effective focus depth of a focusing system.

Similarly, it is advantageous to extend the window of the sample compartment in a direction parallel to the array of detection elements, thus increasing the effective area of the sample being exposed to the array of detection elements.

Using a tubular sample compartment, it is also possible to increase the number of particles being analysed simultaneously by increasing the radius of such tubular sample compartment. The optimum radius of such sample compartment is often determined by the arrangement of the various components of the system, such as focus depth.

As mentioned above, the focus depth of the system, is often important for the determination of optimal dimensions of a sample compartment. Surprisingly it was found that it was possible to use dimension which exceeded the focus depth of a focusing system, even to an extend where the dimension was 2 times or more the effective focus depth, and even 6 times or more.

In the present specification and claims, the term "focus depth" designates the distance an object can move along the axis of a focusing system, without its image is distorted, such distortion being defined as when an image, which when in focus illuminates a single detection element, illuminates an area extending to 2 detection elements in one or two directions, when distorted. When two or more detection elements are combined prior to analysis, the combined detection elements should be considered in the definition of focus depth.

The aspect ratio of a window region of the sample compartment can vary from about 1/1 to 1/100 or even to 1/1000, depending on a focusing method, or other aspects of other components of the system.

The area of the exposing window can be as little as 0.01 mm^2, or as big as 1 mm^2, or even as big as 100 mm^2 or 10000 mm^2. The optimal are of the window often being defined by one or more aspects of this invention.

Generally the volume of the sample being analysed should be as large as possible, preferably 0.01 µl or more, or even 1 µl or more or even as high as 400 µl or more. This allows the simultaneous assessment of a higher number of particles, but the optimal volume is often defined by one or more aspects of this invention.

Often it is preferred to analyse a sample of a sample material without substantially any modification of the sample in full or in part. Other conditions are favoured by imposing one or more modification upon the sample prior to measurement, for instance by removing interfering components or phenomena, or by allowing some modification of a particle or a part of a particle prior to measurement.

Often the particles in question exhibits properties which facilitate the detection of a signal which can be used for the assessment, but sometimes it is preferred to add one or more type of molecules in order to enhance or facilitate any detection of a signal. The number of different types of molecules added depends on the complexity of the assessment, and on the nature of the particles and sample material being analysed. It is for instance often advantageous to use two or more molecules when the assessment concerns the identification of two or more types of particles, where the different particles interact differently with the different molecules, for instance by giving rise to a fluorescent signal at different wavelength. Often the addition of such two or more types of molecules is done simultaneously, but under some conditions it is preferred to add the molecules at different times, preferably in such a way that one or more measurements are carried out between the addition of molecules.

When performing a quantitative assessment of particles it is normally necessary to control the addition of any component to the sample, in order not to affect the result of the assessment. The present invention offers embodiments where such requirements are less important than under conventional situations. This can be accomplished by introducing the components on a form which has only limited effect on the assessment, such as introducing any component as solid matter, thereby substantially not altering the volume of any sample being analysed, even though the final concentration of any added component displays considerable variation.

In a preferred embodiment of the invention the particles being assessed are substantially at stand-still during measurement, thus allowing the optimal use of measurement time in order to improve any signal to noise conditions. This arrangement also eliminates any error which could be inherent in the assessment of particles caused by variation in flow conditions, particularly when an assessment of a property is a volume related property such as the counting of particles in a volume of sample.

In other preferred embodiments the particles are moving during measurement, thus producing the image of a moving particle on the array of detection elements. This can offer advantage in the assessment of particles, especially when any image of the movement can be used for the identification of a particle. Such movement of image

can be homogeneous throughout the array of detection elements, or it can be varying for instance depending on the position of the particle within the sample compartment.

It is also possible to have movements of image, consisting of more than one directional component, which can give advantage when it is necessary to distinct a particle travelling in a predefined way, from a background signal which is substantially random.

When a sample compartment is substantially mechanically fixed in a measuring system, it is an advantage to make use of a method of flow system, which is capable to flow the sample, and/or any other liquid or component into the sample compartment through an inlet, and out of the sample compartment through an outlet, possibly using the inlet for outlet and thereby reducing the complexity of any flow system. Any such flow is often controlled by the use of one or more valves which can control the flow of sample or any other component.

When other components are added to the sample this can be accomplished by means of a flow system which can mix two or more streams of liquid.

After any measurement has been carried out, it is preferred that any sample, or other component used being directed to a waste reservoir.

Another aspect of the present invention, which is particularly of interest when the sample, or any component added to the sample can be considered hazardous, or difficult to handle, is the use of a removable sample compartment. Such sample compartment is readily removed from the measuring system, allowing another sample compartment to take its place. Preferably such sample compartments can be reused or regenerated, maybe after rinsing.

One interesting aspect of a replaceable sample compartment is the possibility of a method for the substantial irreversible closing of the sample compartment after the addition of a sample or any other components, thus preventing any accidental spill or leakage from the sample compartment during storing or transport.

Such sample compartment can be formed in such a way that it comprises more than one compartments where a portion of the same sample material, or portions of different sample material, or portion of other components can be placed. This can for instance allow controlled mixing of liquids.

A sample compartment with more than one compartment could also allow the analysis of more than one portion of the same sample material, or the analysis of more than one sample materials by allowing the different compartment to be exposed to the array of detection elements.

One aspect of such removable sample compartment is that more than one portions of the same sample material can be subjected to analysis by exposure to the array of detection elements. This can be done by allowing the sample compartment to be moved, thus exposing a different portion of the sample compartment, or by allowing the sample within the sample compartment to flow and thereby substantially replace any sample volume exposed with a different sample volume.

This invention offers also methods for the assessment of particles in a removable sample compartment, where more than one such sample compartments are loaded with sample and placed in a transport means which can move the different sample compartment in a position which allows exposure of signals to the array of detection elements. This allows substantial automation of the assessment of particles since more than one sample can be handled at once.

In order to allow optimal assessment of particles it is possible to base such assessment on a number of measurements which are taken from a sample. One advantage is to make repeated measurement of the same portion of a sample, thus improving any signal to noise condition by the use of propagation of error. Another aspect is to increase the total volume of sample which is analysed by taking more than one measurements from different portion of a sample.

Under some conditions it can be advantageous to adjust the measurement time, for instance when the intensity of signals is varying, maybe depending on which type of particles are being analysed.

The present invention offers methods for the assessment of the number of biological particles in a sample with total error, expressed as relative prediction error in per cent of the average number of particles in a volume, which preferably is less than 30 % and often as low as 10 % or even as low or less than 1 %. This can be obtained for instance by controlling the volume of sample which is analysed.

The method of the present invention allow the assessment of biological particles at a rate which preferably amounts to 10 or more assessments per hour, and even as many as 100 or more assessments per hour, even as many as 1000 or more assessments per hour.

It is also possible to combine more than one more or less identical analysing systems, in such a way that they work in parallel and thereby constitute a method which can assess even higher number of samples per hour.

The extensive flexibility of the method of the present invention, makes it possible to analyse volumes, which can allow the assessment of particles in samples where the total number of such particles per volume is ranging from more than $1x10^8$ particles

per ml sample to less and 1 particle per ml sample. One of the most important aspect for that purpose being the total volume being analysed.

The signals which the assessment of particles can be based on are virtually any type of electromagnetic radiation, and in particular where the source of such electromagnetic radiation or mechanism having influence of it can be photoluminescence with lifetime of the exited state of less than or equal to 10^{-6} seconds, photoluminescence with lifetime of the exited state of garter than 10^{-6} seconds, chemiluminescence, rayleigh scatter, raman scatter, attenuation of electromagnetic radiation, absorption of the electromagnetic radiation, scatter of the electromagnetic radiation.

When the signal being detected is an electromagnetic radiation it is preferred to use a detection element which is sensitive to such radiation. Preferred embodiments use arrays of detection elements which are sensitive to electromagnetic radiation of wavelength in one or several of the following regions: 200 nm to 600 nm, 300 nm to 700 nm, 400 nm to 800 nm, 600 nm to 1 μm, 800 nm to 2 μm, 2 μm to 10 μm, 5 μm to 10 μm, 10 μm to 20 μm, 20 μm to 40 μm.

It is often of interest to separate electromagnetic radiation into separate wavelengths or wavebands, especially when the source of such radiation emits energy over a broad spectrum of wavelengths. In methods of assessment of particles which are based on attenuation of energy, detection of emitted or scattered energy or the like, the ability of being able to separate energy into separate wavelengths or wavebands, is important. This applies also to any electromagnetic radiation which originates from within the sample, for instance by means of photoluminescence or chemiluminsecence. In some methods of this invention it is possible to use information obtained when using two or more different wavelengths or wavebands, for instance to distinguish between two or more types of particles on the bases of how those react to different energies.

One method of performing such wavelength separation is to illuminate a portion of a sample with more than one wavelength or waveband simultaneously, preferably in such a way that different portions of the sample are illuminated with different wavelengths or wavebands of energy. This is particularly of interest when the assessment of particles concerns the identification of one or more types of particles since particles are exposed to different wavelength energy depending on their position within the sample compartment.

Another method of performing such wavelength separation is to separate any electromagnetic radiation emitted from the sample, preferably where more than two detection elements observe signals from substantially the same portion of the sample

but due to the wavelength separation these detection elements detect different wavelengths or wavebands of energy. Thereby it is possible to derive spectral characteristics of any particle which can be used for its assessment.

One method is the intensity modulation of electromagnetic radiation. When such modulation is controlled it is possible to use it for the improvement of signal to noise ratio, for instance by observing any background signal in a period where the intensity is low or zero, and then correcting any signal measured when the intensity is high, by the background information.

It is also possible to frequency modulate electromagnetic radiation by the use of an optically active crystals or by the use of an interferometer. The effect of such modulation could be to obtain spectral information about any particle present in the sample.

In methods based on attenuation of electromagnetic radiation, or illumination of the sample it is preferred to use a source of radiation such as light emitting diodes, lasers, laser diodes, thermal light source or gas discharge lamp. When the intensity of the illuminating energy is of interest it is possible to use more than one energy source, even when the different light sources have different energy spectrum, for instance when two different light emitting diodes are used which emit energy in different wavebands.

To improve the efficiency of such light source in illuminating the sample it is often desirable to use a focusing system for focusing energy onto the sample.

When the electromagnetic radiation is used to illuminate a sample for the purpose of causing photoluminescence or the like, it is of interest to be able to increase the efficiency of such radiation source by being able to reflect any energy which is transmitted through the sample back onto the sample, preferably by the use of a reflecting means, for instance dichroic mirrors, which reflect energy of certain wavelengths while allowing the transmission of energies at other wavelengths.

In a method of this invention using photoluminescence as sources of detected signal, it is possible to arrange the light source, relative to the axis of the sample compartment, and particularly relative to an axis which the array of detection elements and the sample compartment form, in such a way that the angle between the axis and the light source is between 0 and 180 degrees.

As detection elements, it is possible to use one of several commercially available arrays of detection elements, such as array of charge coupled devices (CCD) or array of light sensitive diodes (CMOS image sensor). Such arrays of detection elements can have on-chip integrated signal condition and/or signal processing facilities.

At least one embodiment makes use of a calculating means, such as a digital computer, which at least can be used for the correction of any measured signal for a systematic or varying bias, for instance by using one or more predetermined variable to adjust the measured signal. The determination of the predefined variable can be done on the bases of values form measured signals of one or more reference element situated close to the element being corrected, or it can be done on the bases of values from one or several of any other measurements.

In particular it is of interest to subtract one of the other measured signals, often one of previously measured signals, from the measured signal, thereby removing any bias effect which also was present in the other measurement. The other measurement can be another measurement of a different portion of the same sample, or a measurement of a different sample.

Such calculating means can also be used to correct a measured signal for variation in sensitivity, by using one or more predefined variable. The determination of the predefined variable can be done on the bases of values form measured signals of one or more reference element situated close to the element being corrected, or it can be done on the bases of values from one or several of any previous measurements.

One suitable method for the correction of measured signals is to subtract one result from an array of detection elements from another results, obtained form a different portion of the same sample, or from a different sample. Such subtraction has the effect of reducing, or removing any systematic variation in baseline levels of the signals from the array of detection elements, caused by dark-current or possibly by particles which are immobilised on the interior of the sample compartment, any assessment being based on substantially only the positive results of such subtraction. If the two measurement originate from different portion of the same sample, then it is also possible to perform assessment on the bases of the result from the subtraction by treating any negative result from the subtraction as positive number, thus effectively performing an assessment of more than one measurement with the same efforts as when a single measurement is used. In this way it is possible to combine the result from more than one subtraction, preferably as many as the actual noise level allows, for instance by keeping the noise in the combined results less than a given fraction of the smallest signals which are used for the assessment of particles.

The present invention is well suited for making use of state of the art image processing in the assessment of particles, for instance when the assessment is the identification of one or more of different types of particles.

Any instrument constructed according to the present invention can be operated on electrical power, such as 110 or 220 V AC, by the use of appropriate transformator

system. A battery or an accumulator can also be used as a source of power, and this is in particular of interest when the instrument is intended for use where the transport of the instrument is required. Such battery or accumulator can also be one which can be recharged, thereby making it possible to regenerate and reuse.

Example 1

Detection of fluorescence signals from Ethidium Bromide (EtBr) bound to DNA in Somatic Cells in Milk at different initial concentration levels of Ethidium Bromide.

The sample material was cow bulk milk, and to three different portions of the same sample material was added the substantially same amount of a buffer solution but containing different amounts of EtBr prior to measurement. The buffer solution was prepared according to the guidelines of International IDF standard 148A:1995 - Method C, concerning Flouro-Opto-Electronic Method in experiment A, and in experiment B the concentration of EtBr it was about 10 % of the prescribed amount, and in experiment C it was about 1 % of the prescribed amount.

Three portions of the sample material were mixed with a buffer solution, in the ratio one part milk to two parts buffer solution, and measured in a set-up described as follows: A halogen lamp (101) of type OSRAM (41890 SP 12V, 20 W, 10 degree reflector) was used as light source emitting electromagnetic radiation onto the sample by the use of collecting lens (102) contained in a sample compartment (104) through an optical filter (103) selectively transmitting light in the waveband between 400 and 550 nm (Ferroperm SWP 550), any fluorescence signal originating from the sample was focused using a lens (105) with collection angle of approximately 10 degrees and producing an image which is approximately 4 times bigger than the source on a two dimensional array of detection elements (107), a CCD of the type Loral Fairchild (CCD 222) after being passed through an optical filter (106) selectively transmitting light in a waveband between 600 and 700 nm (Schott OG590 and KG5, t=3mm). The final concentration of EtBr in each experiment and the operation of the light source and the detector elements was as follows:

Experiment	EtBr (µg/ml)	Lamp (Volt)	CCD Integration time (ms)
A	33	12	800
B	3.3	12	800
C	0.33	13	1600

The data from the two dimensional array of detection elements was digitised and collected on a computer (not shown) for later analysis.

Results

Data from the two dimensional array of detection elements was used to produce images illustrating measured signals from somatic cells, and an illustration of typical signals from each experiment is shown in Fig. 2, where Fig. 2A is an image of somatic cells as observed in experiment A, Fig. 2B is an image of somatic cells as observed in experiment B, and Fig. 2C is an image of somatic cells as observed in experiment C. In all cases the figures are the numerically positive result of the subtraction of one measurement from the sample, from another measurement of a different portion of the sample, by the formula: $Signal_{(ij)} = ABS(meas1_{(ij)}-meas2_{(ij)})$, where i and j refer to the column and row of the CCD, thus suppressing any systematic bias of the measurement system.

In experiment A, illustrated in Fig. 2A, the signal intensity was such that a majority of the cells shows signals which caused charge overflow on the CCD, resulting firstly in the cut-off of the signal due to the fact that the signal was outside the range of the detector elements, and secondly in broadening of the signal top due to charge transfer from overloaded detection elements to neighbouring detection elements. In addition it is obvious that the signal of the background is considerably high, presumably due to interaction between free EtBr and the sample matrix.

Fig. 2B illustrates typical signals as observed in experiment B. Result A and B were identical apart from the concentration of EtBr used, and the effect on the signal intensity, and signal broadening is evident. In addition the random variation in the background is about 1/2 of the variation observed in experiment A.

Fig. 2C illustrates typical results from experiment C. In this experiment the intensity of excitation light was increased as well as the integration time of the detection elements. The result from experiment C are that the signals are considerably weaker than in experiment B, with a background signal of similar magnitude.

Conclusion

The above illustrates that it is possible to detect signals from somatic cells using concentrations of EtBr, which are considerably lower than concentrations normally used for the fluorescence detection of DNA containing particles. One preferred embodiment of this invention is based on an optical system which has a collection angel of between 40 and 70 degrees, compared to 10 degrees as used in the present example, and this will result in the collection of approximately 10 to 300 times as much energy, making it possible to reduce the concentration of the reagent even further.

Example 2

Removal of signal bias by combination of measurements from a linear array of detection elements.

Removal of systematic signal bias can be of interest in the processing of measured signals. In the present example a linear array of detection elements of the type Hamamatsu (S3902-128Q) was used in an arrangement similar to the one illustrated in Fig. 1. Under the conditions used, the array of detection elements gave readout which had a systematic bias between detection elements with even index and detection elements with odd index. A series of 2 measurements was carried out using water as sample material.

Results

Fig. 3 shows the results of the measurements of water, such that Fig. 3A shows the result from the first measurement after the measurement was adjusted for the mean bias. From Fig. 3A it is apparent that there is a clear difference in the signal intensity of odd and even detection elements, such that elements with odd index have generally lower signal. Fig. 3B shows the result of scan 1 after the results from scan 2 have been subtracted. What is apparent is that the systematic effect of odd and even detection elements is substantially removed, resulting in a signal with a baseline which can be expected to have variations of more random character, the amplitude of this noise can be expected to have an amplitude of approximately 1.41 the amplitude of any random noise present in one measurement.

Conclusion

The conclusion from the above is that it is possible to remove a systematic bias by subtracting one measurement from another.

Example 3

Optical configuration for wide angle collection of signal from a sample

It can be demonstrated that the intensity of any signal collected from a sample is dependent on the square of the collection angle. In the present example two different optical arrangement are used to obtain a collection angle of approximately 40 and approximately 70 degrees respectively.

Fig. 4 illustrates an optical arrangement which produces a collection angle of approximately 40 degrees, when collecting a signal from a sample compartment (401) and projecting it onto detection elements (404), by using two achromatic lenses, one (402) of the type Melles Griot 01 (LAO 014: F=21mm, D=14mm) and another (403) of the type Melles Griot 01 (LAO 111: F=80mm, D=18mm).

Fig. 5 illustrates an optical arrangement which produces a collection angle of approximately 70 degrees, when collecting a signal from a sample compartment (501) and projecting it onto detection elements (506), by using one immersionsobject (502) with radius of approximately 5 mm and width of approximately 8.3 mm, and one aplanatic meniscus lens (503) with one radius of approximately 12.5 mm and one radius of approximately 10.5 mm, and two identical achromatic lenses (504 and 505) of the type Melles Griot 01 (LAO 028: F=31mm, D=17.5mm).

Claims

We claim:

1. A method for the assessment of biological particles in a volume of liquid sample material, by arranging a sample of the liquid sample material in a sample compartment having a wall defining an exposing area, the wall allowing electromagnetic signals from the sample to pass through the wall and to be exposed to the exterior,

forming an image of electromagnetic signals from the sample in the sample compartment on an array of detection elements,

processing the image on the array of detection elements in such a manner that signals from the biological particles are identified as distinct from the sample background,

and, based on the signals from the biological particles identified assessing biological particles in a volume of liquid sample material.

2. A method according to claim 1, where a sample of the sample material contains at least substantially the same components as the sample material.

3. A method according to claim 1 or claim 2 where the liquid sample material is a biological sample material.

4. A method according to any of the preceding claims, with the proviso that when the biological particles are not selected from biological particles somatic cells, red blood cells, blood platelets, bacteria, then the particles are not illuminated from a particle fluorescence-causing illumination source in a direction approximately perpendicular to a light path from the optical cell to the array of detection elements.

5. A method according to any of the preceding claims, where the array of detection elements is arranged in such a way that the detection elements form a substantially straight line.

6. A method according to any of the preceding claims, where the array of detection elements is arranged in two directions in such a way that the detection elements form a series of substantially parallel straight lines.

7. A method according to any of the preceding claims, where the array of detection elements is substantially arranged in one plane.

8. A method according to any of the preceding claims, where the plane of array of detection elements is arranged substantially parallel to an inner boundary of the sample compartment.

9. A method according to any of the preceding claims, wherein the signal from the detection elements is transformed to another measurable signal.

10. A method according to any of the preceding claims, wherein the signal from the detection elements is corrected for signal bias prior to measurement.

11. A method according to claim 10, wherein the bias is estimated from signals from one or more reference detection elements which are situated close to the detection element.

12. A method according to any of the preceding claims, wherein the signal from the detection elements is adjusted for signal sensitivity.

13. A method according to claim 12 , wherein the adjustment of the signal sensitivity is based on one or more of previous measurements.

14. A method according to any of the preceding claims, wherein the signals from more than one detection elements are combined into one measurable signal.

15. A method according to any of any of the preceding claims, wherein the level of 2, preferably 3, more preferably 4, more preferably 5, more preferably 6, more preferably 7, more preferably 8, more preferably more than 8, separate output channels is adjusted in such a way that one, preferably more than one, of the output channels has/have substantially different level from the other output channel(s), where the identification of which of the output channels, or combination thereof, has substantially different output level, is correlated to the intensity of said signal.

16. A method according to any of the preceding claims, where signals from at least a portion of the sample are focused onto the array of detection elements, by the use of a focusing mean, preferably by the use of one lens, more preferably by the use of two lenses, more preferably by the use of more than two lenses.

17. A method according to claim 16 where the ratio of the size of a biological particle, to the size of the image of the biological particle on the array of detection elements is 1/1 or less, preferably less than 1/1 and higher than 1/100, more preferably less than 1/1 and higher than 1/40, more preferably less than 1/1 and higher than 1/10, more preferably less than 1/1 and higher than 1/4, more preferably less than 1/1 and higher than 1/2.

18. A method according to claim 16 where the ratio of the size of a biological particle, to the size of the image of the biological particle on the array of detection elements is in the interval between 5/10 and 20/10, preferably in the interval between 6/10 and 18/10, more preferably in the interval between 7/10 and 16/10, more preferably in tl.e interval between 8/10 and 14/10, more preferably in the interval between 9/10 and 12/10, more preferably substantially equal to 10/10.

19. A method according to claim 16 where the ratio of the size of a biological particle, to the size of the image of the biological particle on the array of detection elements is 1/1 or less, preferably less than 1/1 and higher than 1/100, more preferably less than 1/1 and higher than 1/40, more preferably less than 1/1 and higher than 1/10, more preferably less than 1/1 and higher than 1/4, more preferably less than 1/1 and higher than 1/2.

20. A method according to claim 16, where the ratio of the shorter to the longer of the two dimensions of the image of a biological particle on the array of detection elements is substantially 1 or less, preferably 1/2 or less, more preferably 1/4 or less, more preferably 1/10 or less, more preferably 1/50 or less, more preferably 1/100 or less, more preferably 1/200 or less, relative to the ratio of the corresponding dimensions of the biological particle.

21. A method according to claim 20, where the ratio of the shorter to the longer of the two dimensions of the image of a biological particle on the array of detection elements is substantially not the same within the area spanned by the array of detection elements.

22. A method according to any of claims 16 through 21 where the collection angle of the focusing mean is 15 degrees or less, preferably more than 15 degrees, more preferably more then 30 degrees, more preferably more than 60 degrees, more preferably more than 90 degrees, more preferably more than 120 degrees, more preferably more than 150 degrees.

23. A method according to any previous claim where the size of the detection elements in the array of detection elements is less than 20 μm^2, preferably less than 10 μm^2, more preferably less than 5 μm^2, more preferably less than 2 μm^2, more preferably less than or equal to 1 μm^2.

24. A method according to claims 1 through 22 where the size of the detection elements in the array of detection elements is greater than or equal to 5000 μm^2, preferably greater than or equal to 2000 μm^2, more preferably greater than or equal to 1000 μm^2, more preferably greater than or equal to 500 μm^2, more preferably greater

than or equal to 200 μm^2, more preferably greater than or equal to 100 and less than 200 μm^2, more preferably greater than or equal to 50 and less than 100 μm^2, more preferably greater than or equal to 20 and less than 50 μm^2.

25. A method according to any previous claim where the ratio of the shorter of the height or the width, to the longer of the height or the width of said detection elements in said array of detection elements is substantially equal or less than 1, preferably less than 1/2, more preferably less than 1/4, more preferably less than 1/10, more preferably less than 1/50, more preferably less than 1/100, more preferably less than 1/200.

26. A method according to any previous claim, wherein the assessment of biological particles in a sample is performed without the use of substantially any storage capacity means being used to store measured signals from the detection elements in the array of detection elements.

27. A method according to any of claims 1 to 25 wherein measured signal from the detection elements in said array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements equivalent to, or less than, the number of detection elements, preferably less than 1/2 the number of detection elements, more preferably less than 1/4 the number of detection elements, more preferably less than 1/8 the number of detection elements, more preferably less than 1/16 the number of detection elements, more preferably less than 1/32 the number of detection elements, more preferably less than 1/64 the number of detection elements, more preferably less than 1/128 the number of detection elements, more preferably less than 1/256 the number of detection elements, more preferably less than 1/512 the number of detection elements, more preferably less than 1/1024 the number of detection elements in the array of detection elements.

28. A method according to claims 1 to 25 wherein measured signal from the detection elements in said array of detection elements is stored by means of storage capacity, the storage capacity being able to store a number of measurements greater than the number of detection elements, preferably equivalent to, or greater than, 2 times the number of detection elements, more preferably equivalent to, or greater than, 4 times the number of detection elements, more preferably equivalent to, or greater than, 8 times the number of detection elements, more preferably equivalent to, or greater than, 16 times the number of detection elements, more preferably equivalent to, or greater than, 32 times the number of detection elements, more preferably equivalent to, or greater than, 64 times the number of detection elements, more preferably equivalent to, or greater than, 128 times the number of detection elements,

more preferably equivalent to, or greater than, 256 times the number of detection elements, more preferably equivalent to, or greater than, 512 times the number of detection elements, more preferably equivalent to, or greater than, 1024 times the number of detection elements in the array of detection elements.

29. A method according to any of the preceding claims where said sample compartment limits the dimension of the sample in one direction, substantially not parallel to the plane of array of detection elements, to a thickness of 20 μm or less, preferably to a thickness of more than 20 μm, more preferably to a thickness of more than 40 μm, more preferably to a thickness of more than 60 μm, more preferably to a thickness of more than 80 μm, more preferably to a thickness of more than 100 μm, more preferably to a thickness of more than 140 μm, more preferably to a thickness of more than 180 μm, more preferably to a thickness of more than 250 μm, more preferably to a thickness of more than 500 μm, more preferably to a thickness of more than 1000 μm.

30. A method according to any of the preceding claims, where the sample compartment limits the dimension of the sample in one direction, said direction being substantially parallel to the array of array of detection elements, the length of said dimension being 1 mm or more, preferably 2 mm or more, more preferably 4 mm or more, more preferably 10 mm or more, more preferably 20 mm or more, more preferably 40 mm or more, more preferably 100 mm or more, more preferably 200 mm or more, more preferably 400 mm or more.

31. A method according to any of the claims 1 through 29, where the sample compartment is a substantially cylindrical tube, the tube having an inner radius of more than 0.01 mm, preferably 0.02 mm or more, more preferably 0.04 mm or more, more preferably 0.1 mm or more, more preferably 0.2 mm or more, more preferably 0.4 mm or more, more preferably 1 mm or more, more preferably 2 mm or more, more preferably 4 mm or more, more preferably 10 mm or more.

32. A method according to any of the preceding claims, where the sample compartment limits the dimension of the sample in at least one direction, substantially not parallel to the plane of array of detection elements, to a thickens which is substantially of the same order as the focusing depth of any focusing means used, preferably such that the thickness is greater than 1 times and less than 1.5 times said focusing depth, more preferably equal to, or greater than 1.5 times and less than 2 times said focusing depth, more preferably equal to, or greater than 2 times and less than 3 times said focusing depth, more preferably equal to, or greater than 3 times and less than 4 times said focusing depth, more preferably equal to, or greater than 4 times

and less than 6 times said focusing depth, more preferably equal to, or greater than 6 times said focusing depth.

33. A method according to any of the preceding claims, where the sample compartment limits the dimension of the sample in two substantially perpendicular directions, the ratio between the shorter of the two dimensions to the longer of the two dimensions being substantially equal to or less than 1/1, preferably less than 1/2, more preferably less than 1/4, more preferably less than 1/10, more preferably less than 1/20, more preferably less than 1/33, more preferably less than 150, more preferably less than 1/100, more preferably less than 1/200, more preferably less than 1/500, more preferably less than 1/1000, more preferably less than 1/2000, more preferably less than 1/4000, more preferably less than 1/10000.

34. A method according to any of the preceding claims, where the sample compartment limits the boundary of the sample in two directions, said directions being substantially not parallel to the array of detection elements the boundary being substantially plane with an area of 0.01 mm^2 or more, preferably with an area of 0.1 mm^2 or more, more preferably with an area of 1 mm^2 or more, preferably with an area of 2 mm^2 or more, preferably with an area of 4 mm^2 or more, preferably with an area of 10 mm^2 or more, preferably with an area of 20 mm^2 or more, preferably with an area of 40 mm^2 or more, more preferably with an area of 100 mm^2 or more, preferably with an area of 200 mm^2 or more, preferably with an area of 400 mm^2 or more, preferably with an area of 1000 mm^2 or more, preferably with an area of 2000 mm^2 or more, preferably with an area of 4000 mm^2 or more, preferably with an area of 10000 mm^2 or more.

35. A method according to any of the preceding claims, where the sample compartment limits the boundary of the sample in three directions, in such a way that the volume of the sample is 0.01 µl or more, preferably 0.02 µl or more, more preferably 0.04 µl or more, more preferably 0.1 µl or more, more preferably 0.2 µl or more, more preferably 0.4 µl or more, more preferably 1 µl or more, more preferably 2 µl or more, more preferably 4 µl or more, more preferably 10 µl or more, more preferably 20 µl or more, more preferably 40 µl or more, more preferably 100 µl or more, more preferably 200 µl or more, more preferably 400 µl or more.

36. A method according to any of the preceding claims, where the sample, or parts of said sample, being analysed has been given a chemical, a mechanical or a physical treatment prior to analysis, said treatment being one or several of following: gravity, centrifugation, filtering, heating, cooling, mixing, sedimentation, solvation, dilution, homogenisation, sonification, crystallisation, chromatography, ion

exchange, electrical field, magnetic field, electromagnetic radiation, the effect of said treatment being enhancement of any signal observed from said sample used in the assessment of biological particles in the sample, and/or suppression of any interfering signal.

37. A method according to any of the preceding claims, where the temperature of the sample can be controlled, either by addition or removal of heat from the sample and the temperature of the sample during measurement of the biological particle containing sample is between 0 °C and 90 °C, more preferable between 5 °C and 90 °C, more preferable between 10 °C and 90 °C, more preferable between 20 °C and 90 °C, more preferable between 25 °C and 90 °C, more preferable between 30 °C and 90 °C, more preferable between 35 °C and 90 °C, more preferable between 40 °C and 90 °C.

38. A method according to any of claims 1 through 36, where the temperature of the sample is substantially controlled by ambient temperature and the temperature of the sample during measurement of the biological particle containing sample is between 0 C and 90 °C, more preferable between 5 °C and 90 °C, more preferable between 10 °C and 90 °C, more preferable between 20 °C and 90 °C, more preferable between 25 °C and 90 °C, more preferable between 30 °C and 90 °C, more preferable between 35 °C and 90 °C, more preferable between 40 °C and 90 °C.

39. A method according to any of the preceding claims, where the signal which is detected originates substantially from 1 type of molecules, preferably a mixture of 2 types of molecules, more preferably a mixture of 3 types of molecules, more preferably a mixture of 4 ore more types of molecules, which are bound to, retained within, interacts with, or is prepelled by, the biological particles which are being detected, said molecules being intentionally added to the sample before or during the measurement, preferably one at a time, more preferably more than one at a time.

40. A method according to claim 39 where at least one of said types of molecules is added to a first sample of a sample material and at least another of said types of molecules is added to a second sample of the sample material, preferably where the number of samples of a sample material is equal or less to the number of said different types of molecules, and where at least one measurement is taken from each sample.

41. A method according to claim 39 or claim 40, where at least one measurement of a sample is taken before at least one of said molecules have been

added to the sample and at least one measurement of the sample is taken after all said types of molecules have been added.

42. A method according to any of claims 39 through 42, where said molecules give rise to one or several of the following phenomena: attenuation of electromagnetic radiation, photoluminescence when illuminated with electromagnetic radiation, scatter of electromagnetic radiation, raman scatter.

43. A method according to any of the preceding claims, where the assessment of biological particles is based on the use of nucleic acid dye as an intentionally added molecule in an amount of more than 30 µg per ml of the sample, more preferable in an amount of less than 30 µg per ml of the sample, more preferable in an amount of less than 20 µg per ml of the sample, more preferable in an amount of less than 10 µg per ml of the sample, more preferable in an amount of less than 5 µg per ml of the sample, more preferable in an amount of less than 2 µg per ml of the sample, more preferable in an amount of less than 1 µg per ml of the sample, more preferable in an amount of less than 0.3 µg per ml of the sample, more preferable in an amount of less than 0.03 µg per ml of the sample, more preferable in an amount of less than 0.003 µg per ml of the sample, more preferable in an amount of less than 0.0003 µg per ml of the sample, said nucleic acid stain being one or several of the following, but not limited to: phenanthridines (e.g. ethidium bromide CAS-1239-45-8, propidium iodide CAS-25535-16-4), acridine dyes (e.g. acridine orange CAS-65-61-2/CAS-10127-02-3), cyanine dyes (e.g. TOTO™-1 iodide CAS#: 143 413-84-7 -Molecular Probes, YO-PRO™-1 iodide CAS#: 152 068-09-2 -Molecular Probes), indoles and imidazoles (e.g. Hoechst 33258 CAS#: 023 491-45-4, Hoechst 33342 CAS#: 023 491-52-3, DAPI CAS#:28718-90-3, DIPI (4',6-(diimidazolin-2-yl)-2-phenylindole)).

44. A method according to any of the preceding claims, where the assessment of biological particles is based on the use of potentiometric membrane dye as an intentionally added molecule in an amount of either more than 30 µg per ml of the sample, or, more preferable in an amount of at the most 30 µg per ml of the sample, more preferable in an amount of less than 20 µg per ml of the sample, more preferable in an amount of less than 10 µg per ml of the sample, more preferable in an amount of less than 5 µg per ml of the sample, more preferable in an amount of less than 2 µg per ml of the sample, more preferable in an amount of less than 1 µg per ml of the sample, more preferable in an amount of less than 0.3 µg per ml of the sample, more preferable in an amount of less than 0.03 µg per ml of the sample, more preferable in an amount of less than 0.003 µg per ml of the sample, more preferable in an amount of less than 0.0003 µg per ml of the sample, said nucleic acid stain being one or several of the following, but not limited to: Rhodamine-123, Oxonol V.

45. A method according to any of the preceding claims, where the variation in the concentration of one or more intentionally added component(s) or one or more intentionally added molecules in a sample, is less than, or equal to 1 %, preferably more than 1 %, more preferably more than 2 %, more preferably more than 5 %, more preferably more than 10 %, more preferably more than 25 %, more preferably more than 50 %, of the average concentration of said component when expressed as 1 standard deviation.

46. A method according to any of the preceding claims, where the sample, within the sample compartment, is substantially at stand still during a at least one active period of measurement.

47. A method according to any of the preceding claims, where the sample, within the sample compartment, moves during at least one active period of measurement, the movement being more than or equal to 50 times, preferably less than 50 times, more preferably less than 20 times, more preferably less than 10 times, more preferably less than 4 times, more preferably less than 2 times, more preferably less than 1 times, more preferably less than 1/2 times, more preferably less than 1/4 times, more preferably less than 1/10 times the size of average object size.

48. A method according to any of the preceding claims, where the image of the sample, on the array of detection elements, is substantially at stand still during at least one active period of measurement.

49. A method according to any of claims 1 through 47, where the image of the sample, on the array of detection elements, moves during at least one active period of measurement, the movement being more than or equal to 50 times, preferably less than 50 times, more preferably less than 20 times, more preferably less than 10 times, more preferably less than 4 times, more preferably less than 2 times, more preferably less than 1 times, more preferably less than 1/2 times, more preferably less than 1/4 times, more preferably less than 1/10 times the size of a one detection element of said array of detection elements.

50. A method according to claim 49 where the movement is substantially not uniform within the portion of said sample being exposed to the array of detection elements.

51. A method according to claim 49 or claim 50, where the movement is substantially in one direction, preferably where the direction of moment is substantially parallel to one of the directions the array of detection elements are arranged in.

52. A method according to any of claims 49 through 50, where the movement is substantially in one direction, preferably where the direction of moment is substantially not parallel to any of the directions the array of detection elements are arranged in.

53. A method according to any of claims 49 through 52, where the direction of the movement, or the velocity of the movement is substantially different in at least two periods of detection.

54. A method according to any of claims 49 through 53, where the movement is brought about by means comprising two or more sources of force, said forces can be applied independent of each other, preferably where at least 1 of said forces is substantially perpendicular to at least 1 of the other said forces, more preferably where at least 1 of said forces is applied during a period of time where the other said forces are not applied, more preferably where at least 1 of said forces is applied substantially at the same time as at least 1 of the other said forces.

55. A method according to claims 49 through 54, where said movement is a result of the movement of one or several of the components of the measurement system relative to the others, said components being: illuminating light source, sample, sample compartment, focusing means, array of detection elements.

56. A method according to claims 49 through 55, where the movement of the sample is produced by a pump, the pump being situated either upstream to the sample compartment or downstream to the sample compartment, the pump being one or several of the following but not limited to: peristaltic pump, piston pump, membrane pump, centrifugal pump.

57. A method according to claims 49 through 55, where the movement is produced by difference in pressure potential across the sample compartment, the pressure difference being created by a pressure reservoir, preferably by introducing the pressure difference substantially simultaneously with the introduction of the sample material, more preferably where the movement is produced by difference in pressure potential across the sample compartment, said pressure difference being created by injecting a mixture of sample material and substantially atmospheric air, more preferably the injection being carried out with a syringe, more preferably the injection being carried out with a disposable syringe.

58. A method according to claims 49 through 57, where the movement is controlled by one or more valves, the valves being controlled in such a way that the operation of the valves are substantially synchronised with the measurement periods.

59. A method according to any of the preceding claims, where the sample is introduced to the sample compartment by the means of a flow system, the flow system comprising inlet where liquid enters into the sample compartment and outlet where liquid exits the sample compartment, preferably where the flow of liquid in the sample compartment is brought about by a pump, said pump being situated either upstream to the sample compartment or downstream to the sample compartment, the pump being one or several of the following but not limited to: peristaltic pump, piston pump, membrane pump, centrifugal pump.

60. A method according to any of the preceding claims, where a flow of liquid in the sample compartment is controlled by one or more valves which can control the rate of flow in the sample compartment.

61. A method according to any of the preceding claims, where the flow system allows the mixing of the sample material with a second liquid, preferably where the second liquid is a mixture of two or more chemical component, or a solution of one or more chemical component.

62. A method according to any of the preceding claims, where the outlet from the sample compartment is directed to a waste reservoir, preferably where the waste reservoir is substantially closed to prevent spilling or evaporation from the reservoir.

63. A method according to any of the preceding claims, where the sample compartment is substantially without a connection to a flow system during analysis, preferably where the sample compartment can be removed from the sensing area between observations, preferably for the purpose of replacing the sample within the sample compartment, more preferably for the purpose of replacing the sample compartment with another sample compartment, preferably containing another sample.

64. A method according to claim 63, where the sample compartment is used for the analysis of limited number of samples or sample materials, preferably less than 10, more preferably less than 5, more preferably less than 2, more preferably only 1, before said removable sample compartment being subjected to emptying and/or rinsing and/or addition of one or more components.

65. A method according to claim 63, where the removable sample compartment can only be used for the measurement of one sample or sample material, preferably by means which substantially irreversibly close any access to the removable sample compartment prior to, during or after analysis, preferably in such a way that any part of the sample material, or any component added to the sample material can not be removed from the removable sample compartment after it has been introduced therein.

66. A method according to claims 63 through 65, where the sample compartment is comprised of material which allows destruction by means such as burning or illumination by electromagnetic radiation, preferably comprised of material which allows regeneration, the regeneration comprising one or several of steps such as emptying the sample compartment for any sample material or any other components, rinsing or washing, removal of one or more physical component of the sample compartment, replacing of one or more physical component of the sample compartment or addition of one or more chemical component.

67. A method according to claims 63 through 66, where the sample compartment comprises one or more compartments where chemical or physical components can be stored, the chemical or physical components being added to any sample present in the sample compartment one at a time or more than one at a time.

68. A method according to claims 63 through 67, where the sample compartment comprises means which allows the analysis of more than one sample material either substantially simultaneously or one at a time, preferably in such a way that the sample compartment comprises more than one compartments, preferably where each of the compartments can be used for the analysis of the sample material.

69. A method according to claims 63 through 68, where more than one portion of a sample material or sample placed within the sample compartment is exposed to an array of detection elements, by moving the sample compartment and thereby exposing a different portion of the sample through a different area of an exposing window, or by causing the sample or the sample material to flow within the sample compartment and thereby replacing the portion of the sample or the sample material which previously was exposed to the array of detection elements with a substantially different portion of the sample or the sample material.

70. A method according to claims 63 through 69, where two or sample compartments are filled with sample material and placed within an instrument capable of at least performing the measurements needed for the assessment of biological particles prior to analysis, the instrument being equipped with transport means which at least allow each sample compartment to be placed in a position which allows exposing any signal onto an array of detection elements for measurements and removing the sample compartment upon completion of the measurement, thus allowing the replacement of the sample compartment with a different sample compartment.

71. A method according to any of the preceding claims, where the assessment of the biological objects is based observation from 2, preferably more than 2 and less than 4, more preferably more than or equal to 4 and less than 8, more preferably more than or equal to 8 and less than 16, more preferably more than or equal to 16 and less than 32, more preferably more than or equal to 32 and less than 64, more preferably more than or equal to 64 and less than 128, more preferably more than or equal to 128 and less than 256, more preferably more than or equal to 256 and less than 512, more preferably more than or equal to 512 and less than 1024, more preferably more than or equal to 1024 measurement periods.

72. A method according toclaim 71, where at least one of the measurement periods is divided up into at least two periods, where in at least one of the periods the array of detection element is substantially exposed with signals from the sample and where in at least one of the periods the array of detection elements are substantially not exposed to signals from the sample, the periods being controlled by means which can activate the transmission of electromagnetic radiation on the sample, or the emission of, or the transmission of electromagnetic radiation from the sample.

73. A method according to claims 71 and 72, where the number of the active periods within a measurement period are 2, preferably 3, more preferably 4, more preferably more than 4 and less than 8, more preferably 8 or more and less than 16, more preferably 16 or more and less than 32, more preferably 32 or more and less than 64, more preferably 64 or more.

74. A method according to claims 71 through 73, where each detection element in the array of detection elements measures signal from substantially the same fraction of the sample in two or more of the active periods within a measurement period.

75. A method according to claims 71 through 74, where each detection element in the array of detection elements measures signal from substantially different fraction of the sample, preferably where no fraction of the sample is measured by more than one detection elements on the array of detection elements, in two or more of the active periods within a measurement period.

76. A method according to claims 71 through 75, where duration of the measurement periods is shorter than or equal to $1x10^{-6}$ seconds, preferably longer than $1x10^{-6}$ seconds and shorter than $1x10^{-5}$ seconds, more preferably longer than $1x10^{-5}$ seconds and shorter than $1x10^{-4}$ seconds, more preferably longer than $1x10^{-4}$ seconds and shorter than $1x10^{-3}$ seconds, more preferably longer than $1x10^{-3}$ seconds and shorter than $1x10^{-2}$ seconds, more preferably longer than $1x10^{-2}$ seconds and shorter

than $1x10^{-1}$ seconds, more preferably longer than $1x10^{-1}$ seconds and shorter than 1 second, more preferably longer than 1 second and shorter than 10 seconds, more preferably longer than 10 seconds.

77. A method according to claims 71 through 76, where duration of all the measurement periods is substantially equal.

78. A method according to claims 71 through 76, where duration of at least 2 said measurement periods is substantially different.

79. A method according to claims 71 through 78, where each detection element in the array of detection elements measures signal from substantially the same fraction of the sample in two or more of the measurement periods.

80. A method according to claims 71 through 78, where each detection element in the array of detection elements measures signal from substantially different fraction of the sample, preferably where no fraction of the sample is measured by more than one detection elements in the array of detection elements, in two or more of the measurement periods.

81. A method according to any of the preceding claims, which can assess the number of biological particles in a sample with total error, expressed as standard prediction error which is more than or equal to 30 %, preferably less than 30 %, more preferably less than 20 %, more preferably less than 10 %, more preferably less than 6 %, more preferably less than 4, more preferably less than 2 %, more preferably less than 1 % of the average value of number of biological particles per volume sample.

82. A method according to any of the preceding claims, where the assessment of biological particles can be carried out at a rate which is less than or equal to 10 assessments per hour, preferably greater than 10 assessments per hour, more preferably greater than 30 assessments per hour, more preferably greater than 50 assessments per hour, more preferably greater than 100 assessments per hour, more preferably greater than 200 assessments per hour, more preferably greater than 300 assessments per hour, more preferably greater than 400 assessments per hour, more preferably greater than 500 assessments per hour, more preferably greater than 600 assessments per hour, more preferably greater than 700 assessments per hour, more preferably greater than 1000 assessments per hour.

83. A method according to any of the preceding claims, which uses 2, preferably 3, more preferably 4, more preferably more than 4 parallel detection

systems for the substantially simultaneous assessment of biological particles in a sample.

84. A method according to any of the preceding claims, where the assessment of biological particles in a sample is carried out when the number of biological particles in the sample material is grater than $1x10^8$, preferably less than or equal to $1x10^8$, more preferably less than $1x10^7$, more preferably less than $1x10^6$, more preferably less than $1x10^5$, more preferably less than $1x10^4$, more preferably less than $1x10^3$, more preferably less than $1x10^2$, more preferably less than 10, more preferably less than 1, more preferably less than 0.1, per ml sample.

85. A method according to any of the preceding claims, where the signal which is detected is substantially caused by one or several of the following: photoluminescence with lifetime of the exited state of less than or equal to 10^{-6} seconds, photoluminescence with lifetime of the exited state of garter than 10^{-6} seconds, chemiluminescence, rayleigh scatter, raman scatter, attenuation of electromagnetic radiation, absorption of the electromagnetic radiation, scatter of the electromagnetic radiation.

86. A method according to any of the preceding claims, where the array of detection elements is sensitive to electromagnetic radiation of wavelength in one or several of the following regions: 200 nm to 600 nm, 300 nm to 700 nm, 400 nm to 800 nm, 600 nm to 1 μm, 800 nm to 2 μm, 2 μm to 10 μm, 5 μm to 10 μm, 10 μm to 20 μm, 20 μm to 40 μm.

87. A method according to any of the preceding claims, where spectrally rich electromagnetic radiation can be separated into substantially 1 wavelength component or waveband which is transmitted onto the sample, preferably into 2 or more wavelength components or wavebands which are transmitted onto the sample, one at a time or two or more simultaneously.

88. A method according to any of the preceding claims, spectrally rich electromagnetic radiation emitted from, or transmitted through the sample is separated into substantially 1 wavelength component or waveband, which is measured by a detection element in said array of detection elements, preferably into 2 or more wavelength components or wavebands, which are measured by a detection element in the array of detection elements, one at a time or two or more simultaneously.

89. A method according to any of the preceding claims, where spectrally rich electromagnetic radiation transmitted onto the sample is spatially separated into a

plurality of wavelength components, in such a way that at least two fractions of the sample, are exposed to substantially different wavelength components.

90. A method according to any of the preceding claims, where spectrally rich electromagnetic radiation emitted from, or transmitted through the sample is spatially separated into a plurality of wavelength components, in such a way that each of the detection elements in the array of detection elements, measuring information from substantially the same fraction of the sample, is exposed to substantially different wavelength components.

91. A method according to claim 89 and claim 90 where the separation of spectrally rich electromagnetic radiation is brought about by one or several of the following, but not limited to: interference filters, coloured filters, an optical grating, a prism, an optically active crystals.

92. A method according to any of the preceding claims, where electromagnetic radiation which is transmitted onto, or emitted from, or transmitted through the sample is intensity modulated.

93. A method according to any of the preceding claims, where electromagnetic radiation which is transmitted onto, or emitted from, or transmitted through the sample is modulated by optically active crystals or interferometry, preferably by the use of a Michelson interferometer, more preferably by the use of an interferometer where at least one reflecting surface can be moved.

94. A method according to any of the preceding claims, where the transmission of electromagnetic radiation onto the sample is accomplished by the use of illuminating means.

95. A method according to claim 94, where the illumination means are 2 or more, preferably 3 or more, more preferably 4 or more, more preferably 6 or more, more preferably 8 or more, more preferably 10 or more, light emitting diodes preferably emitting electromagnetic radiation of substantially the same wavelength band.

96. A method according to claim 94 and claim 95, where the electromagnetic radiation transmitted onto the sample is focused by a focusing mean, the focusing mean having the effect of substantially increasing the intensity of said electromagnetic radiation in or at said sample.

97. A method according to any of the claims 94 through 96, where the electromagnetic radiation transmitted onto the sample is accomplished by two or more illuminating means, at least two of the illuminating means having substantially different radiation properties in at least one waveband, the illuminating means being operated in such a way that all transmit substantially simultaneously, preferably at least one of the illuminating means transmitting while at least one other of the illuminating means is not transmitting, more preferably where only one of the illuminating means is transmitting at a time.

98. A method according to any of the claims 94 through 97, where the illuminating means are one or several of the following, but not limited to: light emitting diodes, lasers, laser diodes, thermal light source, gas discharge lamp.

99. A method according to any of claims 94 through 98, where at least a portion of electromagnetic radiation which is transmitted through a sample is reflected back onto or through the sample by the use of a reflecting means, preferably including reflectance means which also can reflect electromagnetic radiation which is scattered or reflected from the boundaries of the sample compartment or the sample is reflected back onto the sample, more preferably where said reflectance means are substantially included in the means which define the boundaries of said sample compartment, preferably where the reflectance mean is one or several dichroic mirrors.

100. A method according to any of claims 94 through 99, where the electromagnetic radiation is transmitted onto said sample from a position which forms an angle which is substantially 0 degrees , preferably between 0 and 15 degrees, more preferably between 14 and 30 degrees, more preferably between 29 and 45 degrees, more preferably between 44 and 60 degrees, more preferably between 59 and 75 degrees, more preferably between 74 and 90 degrees, from the direction between said sample and said array of detection elements.

101. A method according to any of claims 94 through 99, where the electromagnetic radiation is transmitted onto said sample from a position which forms an angle which is substantially 90 degrees, from the direction between said sample and said array of detection elements.

102. A method according to any of claims 94 through 99, where the electromagnetic radiation is transmitted onto said sample from a position which forms an angle which is between 106 and 90 degrees, preferably between 121 and 105 degrees, more preferably between 136 and 120 degrees, more preferably between 151 and 135 degrees, more preferably between 166 and 150 degrees, more preferably

between 180 and 165 degrees, more preferably substantially 180 degrees, from the direction between said sample and said array of detection elements.

103. A method according to any of the preceding claims, where said array of detection elements is one or several of the following types: full frame CCD, frame transfer CCD, interline transfer CCD, line scan CCD.

104. A method according to any of claims 1 through 102, where said array of detection elements is a CMOS image sensor, preferably a CMOS image sensor with on-chip integrated signal condition and/or signal processing, more preferably a CMOS image sensor with on-chip integrated computing means capable of performing image processing.

105. A method according to any of the preceding claims, where a measured signal from one or more detection elements is corrected for systematic or varying bias by the use of a calculating means, the bias correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

106. A method according to claim 105 where the bias correction is performed by subtracting the results obtained in one or several of other measurements from the measured signal, preferably where the other measurements are one or several of measurements of the same sample, or sample material, more preferably where the other measurement is the measurement taken previously of the same sample or sample material.

107. A method according to any of the preceding claims, where a measured signal from one or more detection elements is corrected for intensity by the use of a calculating means, said correction being accomplished by the use of one or more pre-defined value(s), preferably where each measured signal for one or more detection elements in said array of detection elements has one or more pre-defined value(s), more preferably where each pre-defined value is determined on the bases of one or more of any previous measurements.

108. A method according to any of the preceding claims, where the assessment of biological particles is done on the bases of two measurements of the same sample, or sample material, where the two measurements are combined by subtracting one of the measurements from the other measurements thereby creating a measurement result where signals occurring in only one of the measurements are represented by either a

positive or negative measurement result, and signals occurring in both measurements are represented by substantially zero measurement result, preferably using only positive measurement results in the assessment of biological particles, more preferably using both positive and negative measurement results in the assessment of biological particles, more preferably using the absolute value of the measurement results in the assessment of biological particles.

109. A method according to claim 108 where two measurement results are combined by simple addition, preferably where three measurement results are combined, more preferably where four measurement results are combined, more preferably where five measurement results are combined, more preferably where six measurement results are combined, more preferably where more than six measurement results are combined, and used in the assessment of biological particles.

110. A method according to any of the preceding claims, where the distinction between signals from particles and signal from sample background is based on substantially simultaneous use of more than 1, preferably 2 or more, more preferably 4 or more, more preferably 8 or more, more preferably 16 or, more preferably 32 or, more preferably 64 or more measured signals and/or bias corrected signals and/or sensitivity corrected signals from said detection elements in said array of detection elements.

111. A method according to any of the preceding claims, where signals from more than 1, preferably more than 4, more preferably 10 or more, more preferably 50 or more, more preferably 100 or more, more preferably 200 or more substantially parallel, substantially straight lines of detection elements are used for substantially simultaneous distinction between signal from particles and signal from sample background, preferably by combining the signals from said substantially straight lines into one array of values, each value being obtained by combining one or more signals from substantially each straight lines of detection elements thus allowing data from two dimensional array of detection elements to be analysed in the same manner as data from one dimensional array of detection elements.

112. A method according to any of the preceding claims, where the result from the distinction between signal from particles and sample background of the number of objects in 1, preferably 2 or more, more preferably 4 or more, more preferably 8 or more line(s) is/are used in the assessment of biological particles in an adjacent line of detection elements.

113. A method according to any of the preceding claims, where the assessment of biological particles in a sample is used to confirm the presence of any predetermined biological particles in said sample.

114. A method according to any of the preceding claims, where the assessment of biological particles is done by subjecting signals from two dimensional array of detection elements to the methods of image processing or image analysis.

115. A method according to any of the preceding claims, where the source of electrical power is a transformer, capable of transforming alternating electrical source with alternating voltage between -150 and 150 volt, or with alternating voltage between -250 and 350 volt, or with alternating voltage between -350 and 350 volt, into substantially direct current voltage.

116. A method according to any of the preceding claims, where the source of electrical power is one of several of: an accumulator, a removable accumulator, a battery, a rechargeable battery.

117. A method according to any of the preceding claims, where the biological particles are somatic cells and the liquid sample material is milk.

118. A method according to any of the claims 1 through 116, where the biological particles are bacteria and the liquid sample material is milk.

119. A method according to any of the claims 1 through 116, where the biological particles are bacteria and the liquid sample material is blood.

120. A method according to any of the claims 1 through 116, where the biological particles are somatic cells and the liquid sample material is blood.

121. A method according to any of the claims 1 through 116, where the biological particles are bacteria and the liquid sample material is urine.

122. A method according to any of the claims 1 through 116, where the biological particles are somatic cells and the liquid sample material is urine.

123. A method according to any of the claims 1 through 116, where the biological particles are bacteria and the liquid sample material is water.

124. A method according to any of the claims 1 through 116, where the biological particles are blood cells and the liquid sample material is blood.

125. A method according to any of the claims 1 through 116, where the biological particles are blood platelets and the liquid sample material is blood.

126. A method according to any of the claims 1 through 116, where the assessment is the determination of the number of somatic cells in a volume of milk or a milk product, the type of the milk or milk product being one or several of the following: cow milk, goats milk, sheep milk, or buffalo milk.

127. A method according to any of the claims 1 through 116, where the assessment is the determination of the number of bacteria in a volume of milk or a milk product, the type of the milk or milk product being one or several of the following: cow milk, goats milk, sheep milk, or buffalo milk.

128. A method according to any of any of the claims 1 through 116, where the assessment is the determination of the types of bacteria in a volume of milk or a milk product, the type of the milk or milk product being one or several of the following: cow milk, goats milk, sheep milk, or buffalo milk.

129. A method according to any of the claims 126 through 128, where said assessment is carried out substantially simultaneously with the milking, preferably by including the system at-line, more preferably by including the system in-line with a milking system.

130. A method according to claim 129, where the results of the assessment are transferred to one or several information storage means, preferably the information storage means also being able to store other information about the milking, more preferably the information storage means also being able to store information about the bulk of milk previously collected.

131. A method according to claim 130, where the information storage means includes means to indicate whether the milk being milked should be directed to one or several of storage facilities or outlet, the indication being based on the assessment of the number of somatic cells per volume, preferably the indication being based on the assessment as well as other information present in the information storage means about milking of individual animals or the bulk of milk, the other information being one or several of, but limited to: conductivity, impedance, temperature, fat content, protein content, lactose content, urea content, citric acid content, ketone content, somatic cell count.

132. A method according to any of claim 131, where the purpose of the direction of any milk being milked to one or several of storage facilities or outlets is to adjust

the properties of any bulk of milk, preferably with regard to the number of somatic cells per volume.

133. A method according to any of claims 126, 127 or 128, where the assessment is carried out after the milking has taken place, preferably the milk being substantially not altered before measurement.

134. A method according to any of claims 126, 127 or 128, where the assessment is carried out after the milking has taken place, the milk being modified before measurement, preferably in such a way that the modification extends the durability of the sample material, the modification being one or several of, but not limited to; addition of chemical which substantially inhibits bacterial growth in the sample material, addition of chemical which substantially inhibits the growth of fungus, addition of chemical which has colouring properties said colouring being used to aid visual identification of the milk.

135. A method according to any of claims 126 through 134, where the assessment is carried out substantially simultaneously with the assessment of the amount of any constituent in said sample material, preferably by using substantially a same portion of the sample material for the assessment, said constituent being one or several of, but not limited to: fat, protein, lactose, urea, citric acid, glucose, ketones.

136. A method according to claim 135, where said assessment of any chemical constituent is based on spectrophotometric measurement, the spectrophotometric measurement being one or several of, but not limited to; mid-infrared attenuation, near-infrared attenuation, visible attenuation, ultra-violet attenuation, photoluminescence, raman scatter.

137. A method according to any of claims 126 through 136, where the sample material is either a milk sample used for heard improvement purposes, or a milk sample used in a payment scheme.

138. A method according to any of claims 1 through 116, where the assessment is the determination of the number of somatic cells in a volume of blood or a blood product, the type of the blood or blood product being one or several of the following: human blood, animal blood, cow blood, goats blood, sheep blood, or buffalo blood.

139. A method according to any of claims 1 through 116, where the assessment is the determination of the number of bacteria in a volume of blood or a blood product, the type of the blood or blood product being one or several of the following: human blood, animal blood, cow blood, goats blood, sheep blood, or buffalo blood.

140. A method according to any of claims 1 through 116, where the assessment is the determination of the types of bacteria in a volume of blood or a blood product, the type of the blood or blood product being one or several of the following: human blood, animal blood, cow blood, goats blood, sheep blood, or buffalo blood.

141. A method according to any of claims 1 through 116, where the assessment is the determination of the number of somatic cells in a volume of urine or a urine product, the type of the urine or urine product being one or several of the following: human urine, animal urine, cow urine, goats urine, sheep urine, or buffalo urine.

142. A method according to any of claims 1 through 116, where the assessment is the determination of the number of bacteria in a volume of urine or a urine product, the type of the urine or urine product being one or several of the following: human urine, animal urine, cow urine, goats urine, sheep urine, or buffalo urine.

143. A method according to any of claims 1 through 116, where the assessment is the determination of the types of bacteria in a volume of urine or a urine product, the type of the urine or urine product being one or several of the following: human urine, animal urine, cow urine, goats urine, sheep urine, or buffalo urine.

144. A method according to any of claims 138 through 143, where the purpose of the assessment is to obtain information about the status of health, such as infection, preferably where the assessment is carried out in medical doctor office, physician office or veterinary office.

145. A method according to any of the preceding claims, where the sample material to be measured is contained in a vial before analysis, preferably where substantially entirely all the sample material used for the assessment along with any components intentionally added to the sample material or portion of the sample material is returned to the vial after the completion of the measurement.

146. A method according to claim 145, where substantially entirely all the sample material used for the assessment along with any components intentionally added to the sample material or portion of the sample material is returned to a vial after the completion of the assessment, preferably the vial being substantially closed to prevent spilling or evaporation of any material contained within the vial, more preferably the vial prior to the addition of any sample material, contains one or more chemical components, the function of the chemical components being one, or several, but not limited to: substantial inhibitation of bacterial growth, substantial inhibitation of growth of fungus.

147. A method according to any of the preceding claims, where the sample being analysed is substantially an aqueous solution or an organic solution.

148. A method according to any of the preceding claims, where the sample being analysed contains two or more phases in suspension, at least one of the phases being inmiscible under the condition the measurements are carried out.

149. A method according to claim 148, where all the phases of the sample are substantially liquid under the condition the measurements are carried out.

150. A method according to claim 148 where at least one of the phases of the sample is/are substantially solid under the condition the measurements are carried out.

151. A method according to any of the preceding claims, where the sample contains material, the material being dissolved and/or suspended, the amount of the material being substantially more than or equal 25 %, preferably less than 25 %, more preferably less than 10 %, more preferably less than 5 %, more preferably less than 1 %, more preferably less than 0.1 %, more preferably less than 0.01 %, more preferably less than 0.001 %, more preferably less than 0.0001 %, more preferably less than 0.00001 %, more preferably less than 0.000001 %, of the total weight of said sample.

152. A method according to any of the preceding claims, where substantially entirely no components have intentionally been added to the sample being analysed.

153. A method according to any of claims 1 through 151, where the sample has been intentionally modified by the addition of 1 solid, liquid, dissolved or suspended component equivalent to more than or equal to 50 %, preferably less than 50 %, more preferably less than 35, more preferably less than 20 %, more preferably less than 10 %, more preferably less than 5 %, more preferably less than 2 %, more preferably less than 1 %, more preferably less than 0.1 %, more preferably less than 0.01 %, more preferably less than 0.001 %, more preferably less than 0.0001 %, more preferably less than 0.00001 %, more preferably less than 0.000001 % of the total weight of the sample.

154. A method according to claim 153, where the addition comprises more than or equal to 10, preferably less than 10, more preferably less than 6, more preferably less than 4, more preferably less than 3 solid, liquid, dissolved, or suspended components.

155. A method according to claim 153 or claim 154, where said addition of said 1 or more components enhances the signal detected from the objects in a sample.

156. A method according to any of the claims 153, 154 or 155, where said addition of said 1 or more components suppresses one or more signals from the sample which is being measured, the signal being a signal interfering with the signal detected from the biological particles in a sample.

157. A method according to any of the claims 153 through 156, where one of intentionally added chemical components has the effect of adjusting the pH of the sample before or during the assessment, the chemical component being one or several of the following, but not limited to: citric acid, citrate, acidic acid, acetate, phosphor acid, phosphate, carbonate, bicarbonate, boric acid, borate.

158. A method according to any of the claims 153 through 157, where one intentionally added chemical components has the effect of adjusting the pH of the sample before or during the assessment, the chemical component being a mixture of citric acid and citrate.

159. A method according to any of the claims 153 through 156, where one intentionally added chemical components has the effect of enhancing any signal detected from the biological particles, the chemical component being a mixture of citric acid and citrate.

160. A method according to any of the claims 153 through 156, where one of intentionally added chemical components has the effect of surfactant, the chemical component being of one or several of the following groups of surfactants, but not limited to: anionic surfactant, cationic surfactant, amphoteric surfactant, nonionic surfactant.

161. A method according to claim 160, where one intentionally added chemical component is t-Octylphenoxypolyethoxyethanol (Triton X-100).

162. A method according to any of the claims 153 through 156, where one of intentionally added chemical components has the effect of binding one or several of metal ions present in the sample, preferably the chemical component being one capable of forming a metal ion complex with the metal ion.

163. A method according to claim 162, where the intentionally added chemical component is one or several of the following, but not limited to: EDTA, Oxalic acid,

Oxalate, Ethylene glycol-bis(ß-aminoethyl ether) N,N,N',N'-tetraacetec acid (EGTA).

164. A method according to any of the preceding claims, where the biological particles to be assessed are one or several of the following, but not limited to: somatic cells, red blood cells, blood platelets, bacteria, yeast cells, fragments of cells, lipid globules, protein micelles, plankton, algae.

165. A method according to any of the preceding claims, where the biological particles to be assessed comprises polymer beads bound to biological particles or components in connection with assessment of these biological molecules or components.

166. A method according to any of the preceding claims, where the sample material is one or several of the following, but not limited to: specimen of human origin, specimen of animal origin, drinking water, waste water, process water, sea water, lake water, river water, ground water, food, feed or components of food and feed, milk or a milk product, blood or a blood product, urine, faeces, saliva, specimen from an inflammation, specimen from the petrochemical industry, specimen from the pharmaceutical industry, specimen from the food or feed industry.

167. A method according to any of the preceding claims, where the average size of the biological particle to be assessed is less than 0.01 µm, preferably less than 0.1 µm, more preferably less than 1 µm, more preferably less than 2 µm, more preferably less than 3 µm, more preferably less than 4 µm, more preferably less than 6 µm, more preferably less than 10 µm, more preferably less than 20 µm, more preferably less than 50 µm, more preferably less than 100 µm.

168. A method according to any of the preceding claims, where the average size of the biological particle to be assessed is larger than or equal to 100 µm, preferably larger than 150 µm, more preferably larger than 200 µm, more preferably larger than 400 µm.

169. A method according to any of the preceding claims, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a milk sample, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

170. A method according claim 169, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

171. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a milk sample, the purpose of the assessment being to obtain information about the health status of a milking animal, preferably to obtain information about subclinical or clinical mastitis, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

172. A method according claim 171, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

173. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a milk sample, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample the time between the replacement of sample material being shorter than 30 seconds, preferably shorter than 15 seconds, more preferably shorter than 10 seconds,, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

174. A method according claim 173, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to

or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

175. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a milk sample, the assessment being performed substantially at the beginning of milking, or during milking, or immediately after milking has taken place, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample flowing milk directly from a milking unit or flowing milk from an intermediate reservoir which is gradually filled during milking, preferably where said reservoir is filled with milk substantially representing the composition of the total volume of milk being milked, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

176. A method according claim 175, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

177. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a milk sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

178. A method according claim 177, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to

or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

179. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a milk sample, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

180. A method according claim 179, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

181. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a milk sample, the purpose of the assessment being to obtain information about the health status of a milking animal, preferably to obtain information about subclinical or clinical mastitis, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

182. A method according claim 181, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

183. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a milk sample, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample the time between the replacement of sample material being shorter

than 30 seconds, preferably shorter than 15 seconds, more preferably shorter than 10 seconds,, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

184. A method according claim 183, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

185. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a milk sample, the assessment being performed substantially at the beginning of milking, or during milking, or immediately after milking has taken place, the sample of the sample material is placed in a sample compartment by the use of a flow means capable of replacing the sample within the sample compartment with a different sample flowing milk directly from a milking unit or flowing milk from an intermediate reservoir which is gradually filled during milking, preferably where said reservoir is filled with milk substantially representing the composition of the total volume of milk being milked, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

186. A method according claim 185, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

187. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a milk sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent

signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

188. A method according claim 187, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

189. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a blood sample, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

190. A method according claim 189, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

191. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a blood sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

192. A method according claim 191, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

193. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a urine sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

194. A method according claim 193, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

195. A method according to any of claims 1 through 168, wherein the biological particles are somatic cells or fragments thereof, and the sample material is a urine sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said somatic cells or portions of said somatic cells or components interacting with or bound to the somatic cells or portions thereof.

196. A method according claim 195, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the somatic cells or parts of the somatic cells, preferably by binding to or interacting with DNA material contained within or originating from the somatic cells.

197. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a urine sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each

of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

198. A method according claim 197, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

199. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a urine sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

200. A method according claim 199, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

201. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a water sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

202. A method according claim 201, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

203. A method according to any of claims 1 through 168, wherein the biological particles are bacteria or fragments thereof, and the sample material is a water sample, a portion of the sample material is placed in a sample compartment being at least a part of a unit which can be replaced between substantially every assessment or where each of said units can only be used for said assessment of one of said sample materials, the sample of the sample material is illuminated in the sample compartment with electromagnetic radiation where at least a portion of said electromagnetic radiation has energy which can give rise to a photoluminescence signal, preferably fluorescent signal, the signal originating at least from said bacteria or portions of said bacteria or components interacting with or bound to the bacteria or portions thereof.

204. A method according claim 203, wherein the signal originates from one or several types of molecules intentionally added to said sample which interact or bind to or interact with the bacteria or parts of the bacteria, preferably by binding to or interacting with DNA material contained within or originating from the bacteria.

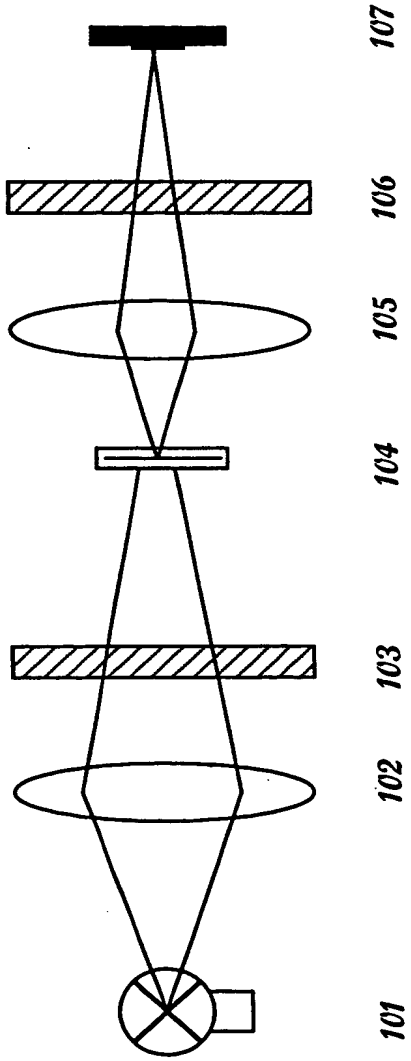
Title

Assessment of biological particles

Abstract

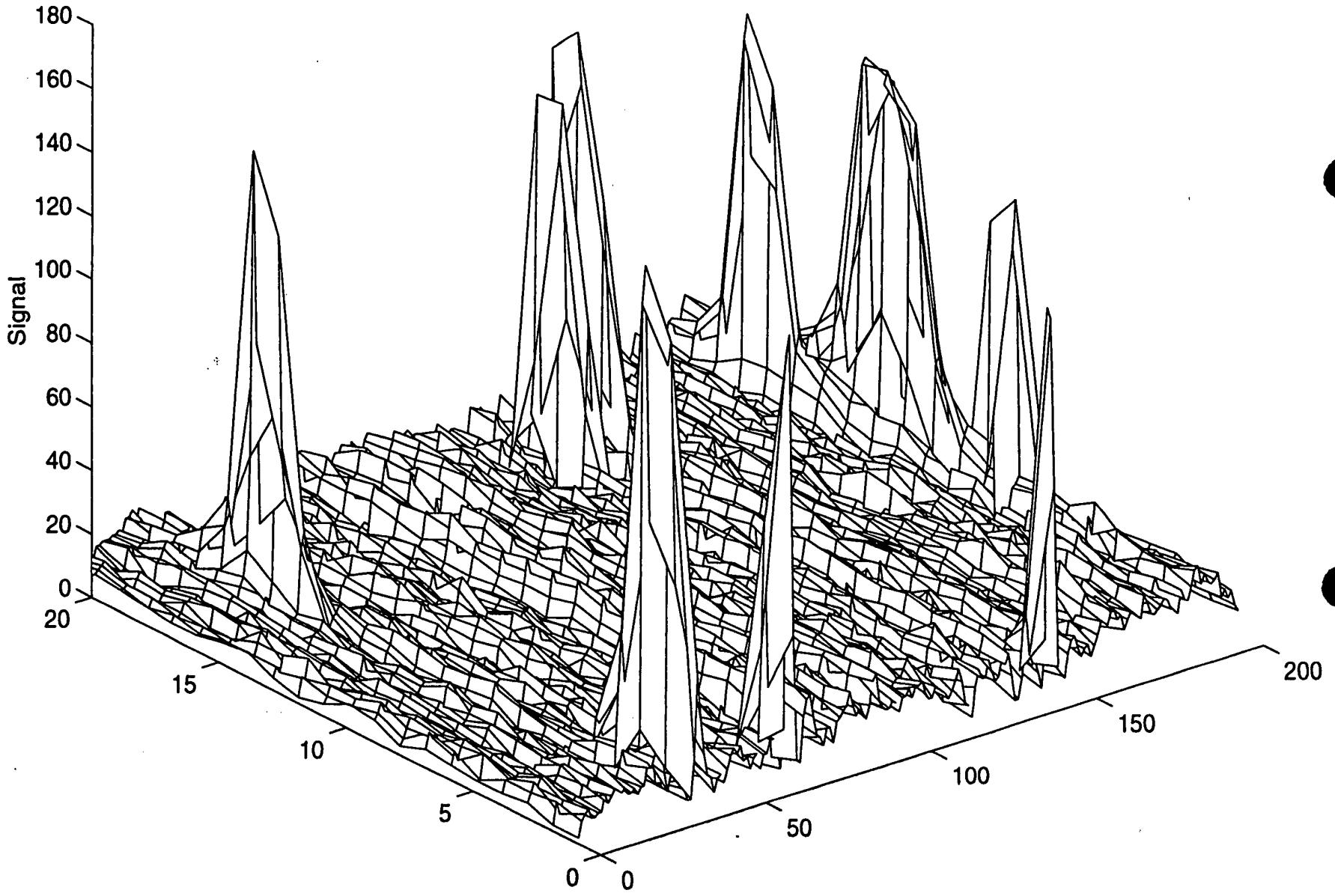
This invention relates to a method for the assessment of the number of particles in a volume of liquid sample material, the method comprising arranging a sample of the liquid sample material in a sample compartment having a wall defining an exposing area, the wall allowing signals from the sample to pass through the wall and to be exposed to the exterior, forming an image of signals from the sample in the sample compartment on an array of detection elements, processing the image on said array of detection elements in such a manner that signals from said particles are identified as distinct from the sample background, and, based on the signals from said particles identified assessing the number of particles in a volume of said liquid sample material.

Fig. 1



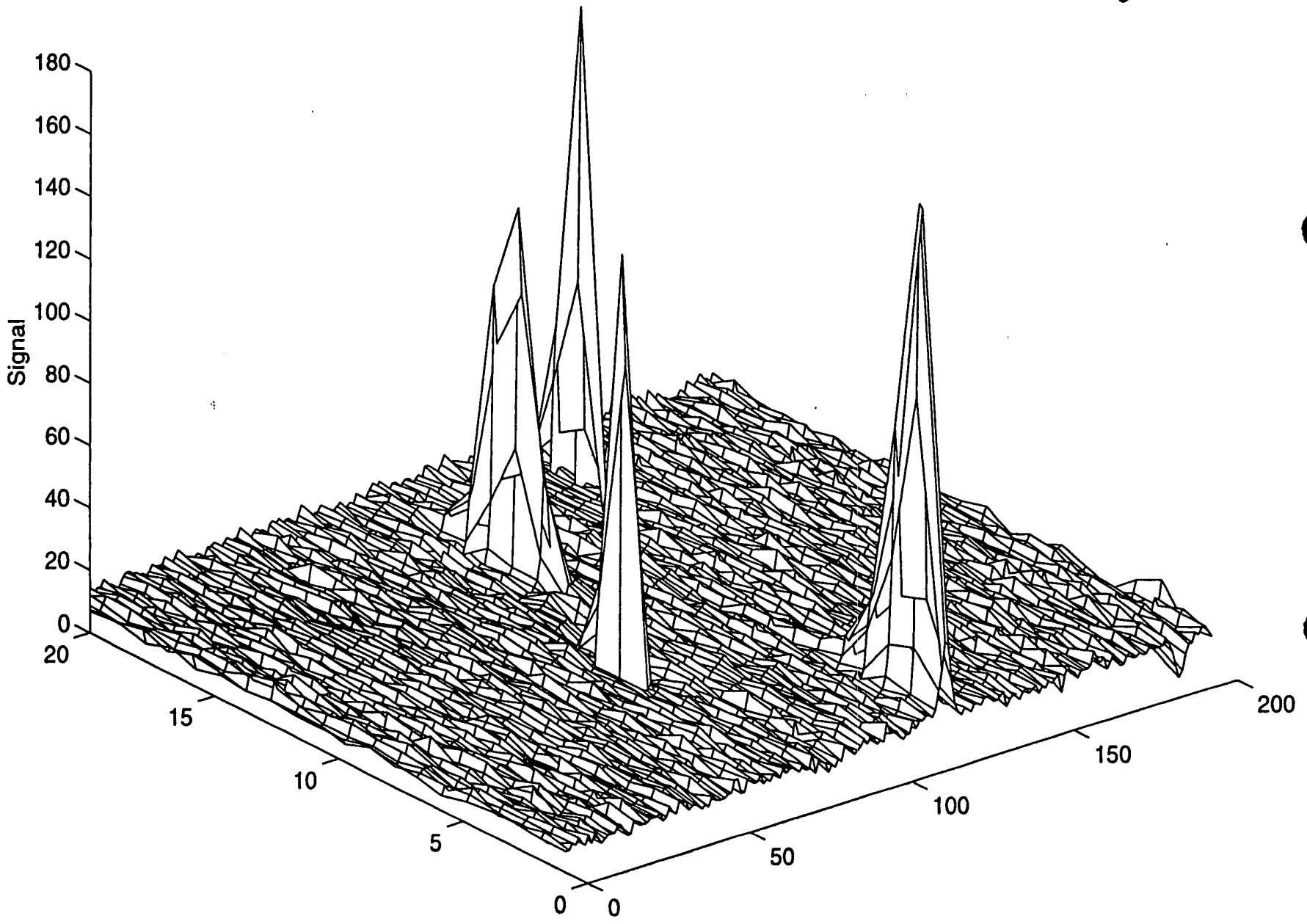
EtBr=33µg/g - ABS(Image #1-#2)

Fig. 2A



EtBr=3.3µg/g - ABS(Image #1-#2)

Fig. 2B



EtBr=0.33µg/g - ABS(Image #1-#2)

Fig. 2C

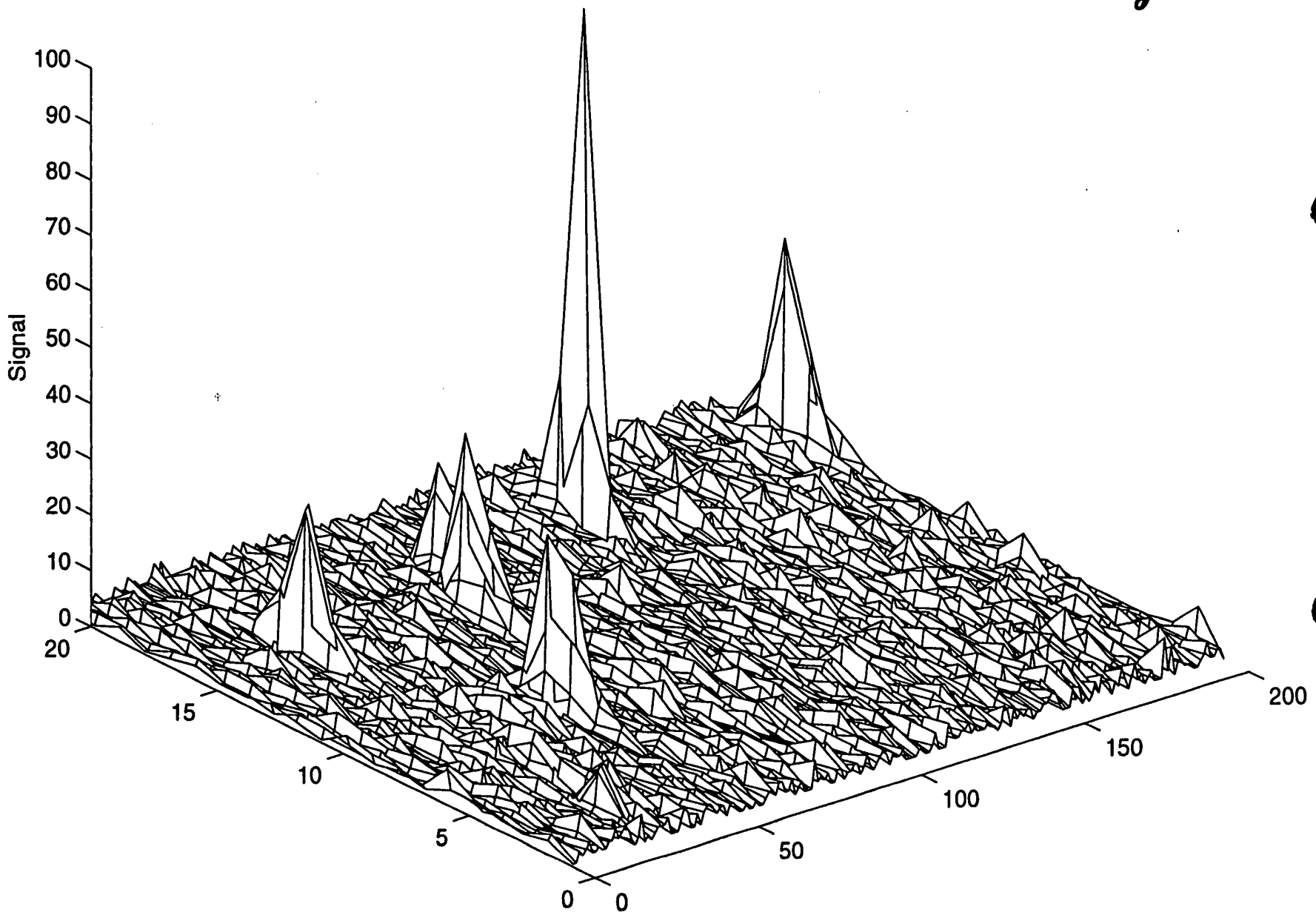


Fig. 3A

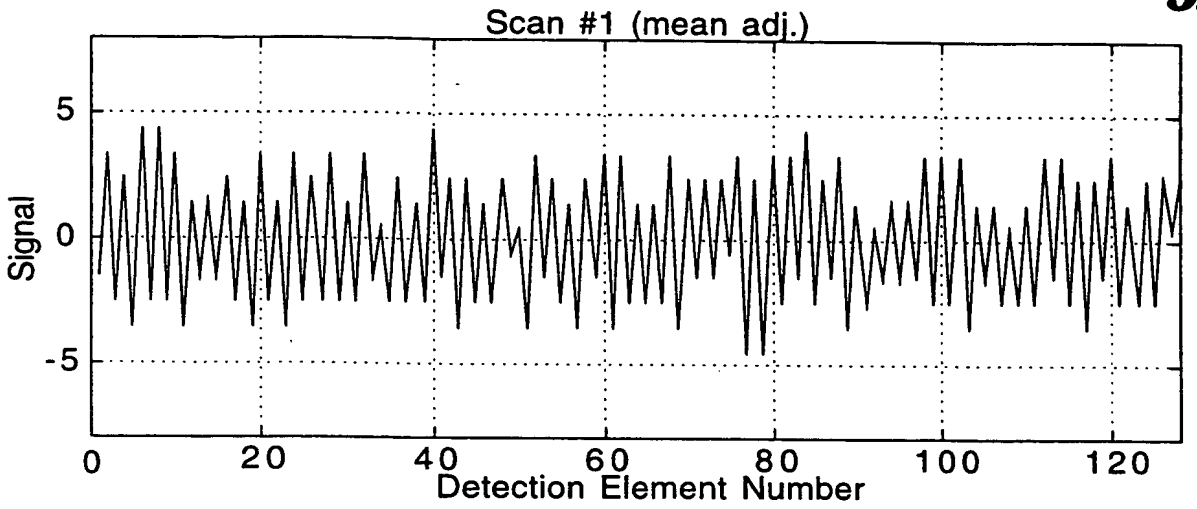

Scan #1 (mean adj.)
Signal
5
0
-5
0
20
40
60
80
100
120
Detection Element Number

Fig. 3B

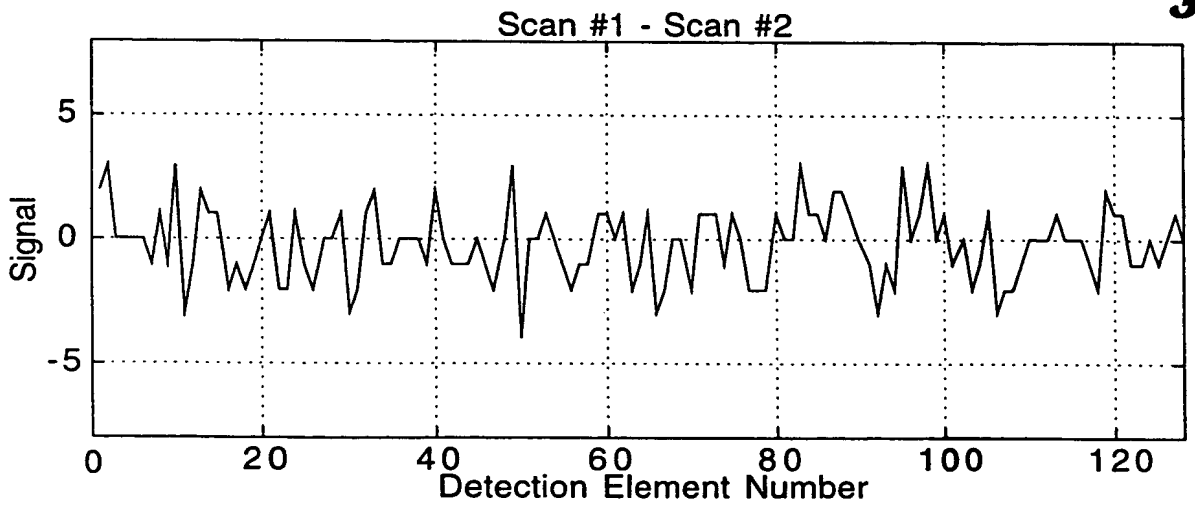

Scan #1 - Scan #2
Signal
5
0
-5
0
20
40
60
80
100
120
Detection Element Number

Fig. 4

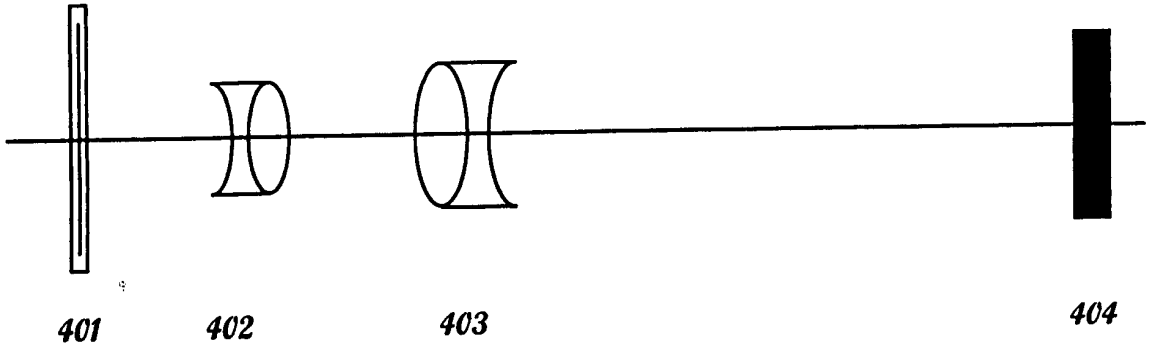


Fig. 5